SECOND DAY

TUESDAY—MORNING SESSION

REPORT ON VITAMINS

By CHESTER D. TOLLE (U. S. Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Referee*

Vitamin A.—The Associate Referee made his report under the title "The Present Status of the Chromatographic Spectrophotometric Procedure for the Estimation of Vitamin A in Margarine."

Vitamin B_1—The Associate Referee made no report.

Vitamin B_2. (*Riboflavin, Microbiological*).—The first recommendation of the Associate Referee, which reads as follows, is approved:

That the microbiological method for the determination of riboflavin described in *This Journal*, **29**:25, 1946, be adopted as tentative and that the methods previously adopted be dropped. (*This Journal* **24**:413; **25**:459; **27**:540).

Vitamin B_2. (*Riboflavin, Chemical*).—The recommendation of the Associate Referee, which reads as follows, is approved:

That the fluorometric method for the determination of riboflavin described in the report of the Associate Referee be studied collaboratively during the coming year.

Vitamin C.—The *Associate Referee* made no report.

Vitamin D. (*Poultry*)—The *Associate Referee* made no report.

Nicotinic Acid.—The recommendation of the Associate Referee, which reads as follows, is approved:

It is recommended that the 1945 U.S.P.—A.O.A.C. method as described* (p.), be made official for the determination of nicotinic acid.

Pantothenic Acid.—The Associate Referee made no report.

Carotene.—The recommendations of the Associate Referee, which read as follows, are approved:

(1) That adsorbents be further studied in order to develop tests by which suitability of an adsorbent for the determination of carotene can be ascertained.

(2) That the methods for carotene previously adopted (*This Journal*, **24**:859; **25**:886; **27**:542; *Methods of Analysis, A.O.A.C.*, p. 369, 1940) be dropped and that the method described in *This Journal*, **29**:18, 1946, for the determination of carotene be adopted as tentative.

Note.—Description of the above methods will be found in the reports of the Associate Referees, *This Journal*, pp. 382 to 412.

* Details of The Method are given in *This Journal*, **30**, 82 (1947).

REPORT ON VITAMIN A

THE PRESENT STATUS OF THE CHROMATOGRAPHIC SPECTROPHOTOMETRIC PROCEDURE FOR THE ESTIMATION OF VITAMIN A IN MARGARINE

By J. B. WILKIE (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Two years ago Wilkie and De Witt presented a paper[1] giving results of a method for estimating the amount of vitamin A in margarine by chromatography and spectrophotometry. Routine results obtainable at that time were quite uniform and provided reasonable values. In general, the method also checked the biological and the antimony trichloride methods although there were noticeable exceptions with the latter method.

With these facts in mind it seemed logical to try some collaborative work with the method.

Accordingly, interested parties were solicited largely through the help of Dr. Rice, Chairman of the Vitamin A Committee of the National Association of Margarine Manufacturers. Collaborators were thus obtained for a study in 1945. The second, or 1946 A.O.A.C. collaborative study, was dovetailed to a certain extent with the current N.A.M.M. antimony trichloride "Blue Color" studies to facilitate the work of both groups.

Two samples of margarine were used for the first, or 1945, A.O.A.C. study. These were designated E and T. E was a common type margarine fortified to contain approximately 12,000 units of vitamin A per pound. T was a highly hydrogenated sample for tropical use containing 12,500 units of vitamin A per pound. The results of this study are summarized in Table 1. It will be observed that a total of 8 laboratories reported work with the method. Probably because of lack of explicitness for the method, few laboratories reported more than 3 or 4 runs with each sample, as shown in the third column. For example, Laboratory number 5, reported only 1 run; Laboratory number 4, 2 runs; and this was true even though good checks between each of the runs was not obtained.

Towards the top of Table 1, the right-hand columns labeled $D\frac{290}{340}$ and $D\frac{325}{340}$ are the averages of the density ratios at the specified wave lengths, 290–325–and 340 being the specified wave lengths in millimicrons. These $D\frac{290}{340}$ and $D\frac{325}{340}$ absorption ratios or data for calculating them were not consistently furnished to analysts. This was a real deficiency, since these ratios now appear to be important indices of certain types of correctable manipulative error. A column labeled Average False Recovery has been

[1] Wilkie, J. B., and DeWitt, J. B., "Spectrophotometric Procedure for the Estimation of Vitamin A in Oleomargarine," *This Journal*, **28**, 174 (1945).

TABLE 1.—*Summary of 1945 A.O.A.C. collaborative data on vitamin A in margarine*

LAB.	SAMPLE	NO. REPD. RUNS	UNITS/LB.			CV ON μ/LB.	PROB. VAL. ON 80% RECOV. BASIS	AV. FALSE RECOV. %	CV ON FALSE RECOV. %	AV. D 290/340	AV. D 325/340	BIO. VAL.
			MINIMUM	MAXIMUM	AVERAGE							
1	E	4	6,800	7,800	7,790	11.6	9,737	41.5	26.2	—	—	9,000
1	T	6	2,060	12,180	7,128	61.0	8,910	43.0	90.2	—	—	9,000
2	E	6	3,859	13,300	6,934	52.0	12,321	55.0	84.3	5.53	1.34	
2	T	6	4,880	11,000	6,834	32.4	8,540	49.0	76.6	4.66	1.41	
4	E	2	12,826	12,939	12,882	16.0	16,200	65.6	5.89	00	1.52	
4	T	3	12,485	14,704	13,317	5.93	16,700	47.0	85.61	00	1.51	
5	E	1	—	—	12,100	—	15,125	—	—	2.28	1.27	
5	T	1	—	—	10,700	—	13,375	—	—	2.84	1.24	
7	E	7	9,352	12,757	10,727	11.2	13,400	—	—	—	1.45	
7	T	4	6,401	12,212	8,648	35.1	10,810	47.2	23.7	—	1.41	
9	E	1			16,117							
11	E	5	7,990	9,715	9,097	7.24	11,372	—	—	—	—	
17	E	3	10,000	11,250	10,187	12.5	12,800	28.7	100.0	3.56	1.30	
17	T	2	9,100	9,900	9,500	5.8	11,800	50.0	—	3.10	1.30	

E—11,000 Vitamin A μ/lb. calculated initial strength. Alcohol in cottonseed oil concentrate used. Average type margarine.
T—100% hydrogenated 103°–105° Wiley M.P. Non-Sap. Conc. used. 12,500 Vitamin A units/lb. actually added.

included. This is the fifth column from the right. For example, Laboratory number 1 has an indicated false recovery of 41.5 per cent for sample E and 43 per cent for sample T. These recoveries are apparent actual recoveries, but the word "false" is most representative since the recoveries are always more variable,and generally excessively too low, for any logical agreement with the actual vitamin A unitage values obtainable. The coefficient of variation (CV) has been chosen as the best index of reproducibility. It will also be noted that there is a column labeled "Probable value on an 80% recovery basis." This column was inserted because this recovery is in the range of actual and most logical values obtained to date

TABLE 2.—*Showing effect of using improper MgO ratio*

LAB.	SAMPLE	UNITS/LB	AV. D 290/340	AV. D 325/340
USING 3 MgO TO 1 CELITE				
5	E	15,200	00	1.48
5	E	18,000	00	2.10
5	T	17,800	00	2.84
5	T	19,300	00	1.98
USING 1 MgO TO 3 CELITE. (A MORE RAPID COLUMN)				
5	E	12,100	*2.28	1.27
5	T	10,700	*2.84	1.24
SP	E	10,025	3.10	1.22
SP	T	9,100	2.25	1.25

* Extrapolated value.

and because special recovery experiments also indicated its use. This will be discussed later.

The average values for vitamin A potency as reported by the different laboratories given in column 6 of Table 1 were used as a basis for calculating the coefficient of variation between laboratories. The CV of less than 16 demonstrates that at least one-half of the laboratories were able to use the method with at least partial success. When all of the results were considered a CV of 34 was obtained. Actually the reasons for these wider discrepancies are now recognized as being due to inadequacy of directions and to manipulative errors. With this knowledge, there seems to be little doubt that errors could have been greatly reduced in the collaborative work.

With sample T the results were more discordant, probably because of its more highly hydrogenated condition.

One of the difficulties that caused erratic results in one of the reporting laboratories was improper preparation of the chromatographic column.

The first part of Table 2 shows these results caused by using 3 parts of magnesium oxide: 1 part of Celite rather than the recommended 3 Celite: 1 MgO mixture. It will be observed that the results are both too high and erratic. The unusually high $D\frac{325}{340}$ ratio is especially noticeable. Values as high as 2.1–2.84 appear in the upper part of Table 2. After correcting the ratio of magnesium oxide to Celite, that is, after using 3 of Celite to 1 of magnesium oxide, the unitage values immediately become logical and the ratio of $D\frac{325}{340}$ also attained normal values of less than 1.3. The similarity of these values with those of another collaborator working with properly prepared columns, are given in the lower part of Table 2.

The experience of this collaborator's laboratory definitely indicates that good results can be attained by proper attention to the details of the method, especially as related to the speed and the activity of the column. The 3 Celite: 1 MgO makes a speedier column than the original used by mistake in the work reported in Table 2.

The first collaborative study thus was completed with the majority of collaborators apparently in position to gain useful control of the method and the others with the method essentially out of control. Several of the collaborators indicated that more detailed instructions might improve the results. Accordingly a quite comprehensive set of instructions involving tests for column performance and other revisions, such as evaporating the extract to dryness to improve the eluting, and a rapid method for purifying petroleum ether before chromatographing, was prepared and sent to collaborators for the 1946 collaborative study. Only six laboratories including our own have reported. Table 3 summarizes the 1946 results. Of the six laboratories reporting, the results of only four can justifiably be considered. Results of Laboratory number 4 were eliminated because of excessively destructive column behavior as evidenced by the report of only 61% recovery of standard, rather than the more than 95% to be expected. Furthermore, the absorption ratios indicated exceedingly poor column separation.

Results from Laboratory 18 were disqualified because the values were too limited, too high, and too variable, also because no ratios were available for the estimation of validity or nature of the difficulties encountered.

To summarize, the four 1946 studies represented improved over-all reproducibility since the over-all coefficient of variation was 14 in comparison with a like group in the 1945 study with a coefficient of variation of 34. But the results still indicated too much of the same kind of difficulty.

Laboratory 7, for example, had two values which were too large. These larger values were accompanied by corresponding excessive $D\frac{325}{340}$ ratios indicating impurities or poor column separation. This is reflected in the value given in the last column in Table 3. The collaborator from Laboratory number 7 stated that a 1:1 Celite-MgO ratio was used. This was likely to result in a slow column, difficult to control. Activity with speed should

TABLE 3.—*Summary of 1946 A.O.A.C. collaborative data on vitamin A in margarine*

LAB.	SAMPLE	NO. REPD.	UNITS/LB.			CV ON μ/LB.	PROB. VALUE ON 80% RECOV. BASIS	AV. FALSE RECOV.	CV ON FALSE RECOV.	AV. D 290/340	AV. D 325/340
			MINIMUM	MAXIMUM	AVERAGE						
								Percent	*Percent*		
18	J	2	16,800	17,400	17,100	13.1	—	—	—	—	—
2	J	4	3,130	13,000	7,617	88.0	9,600	114.0	46.0	5.70	1.37
7	J	6	11,509	19,408	15,017	20.8	18,800	72.6	20.6	—	1.50
19	J	5	10,000	14,000	11,820	15.9	15,000	23.5	—	4.50	1.36
4	J	2	12,485	14,755	13,620	11.7	17,000	85.0	7.05	00	1.65
17	J	2	15,000	16,500	15,750	7.8	19,700	—	—	3.43	1.43

be attainable with 3 parts of Celite to 1 part of MgO, or 6 parts of Celite to 1 part of MgO column.

The average vitamin A value reported by Collaborator 7 was brought to a more reasonable value because the other runs were undoubtedly more correct and were accompanied by lower $D\frac{325}{340}$ ratios indicating the fact. The unitage values from Laboratory 19 were low and accompanied by a high $D\frac{290}{340}$ ratio indicating excessive deterioration possibly caused by too slow a column. The column should be both active (as determined by special tests or actual runs) and fast. It has been our experience that columns

TABLE 4.—*Reproducibility of 1946 method in food and drug vitamin laboratory*

DATE	SAMPLE	μ/LB	PROB. VALUE ON 80% RECOV. BASIS	FALSE RECOVERY	AV. D 290/340		AV. D 325/340	
					REG. RUN	RECOV. RUN	REG. RUN	RECOV. RUN
				%				
1945								
9–25	T	8,800	11,000	—	3.78	—	1.30	—
9–25	T	8,800	11,000	—	3.78	—	1.40	—
9–26	T	8,100	10,125	92.5	3.94	3.20	1.37	1.30
9–26	T	8,100	10,125	65.0	3.18	2.50	1.37	1.34
10–1	T	9,050	11,312	60.0	3.51	2.76	1.35	1.34
10–2	T	8,500	10,600	65.0	4.00	4.45	1.36	1.32
10–2	T	8,500	10,600	67.0	4.00	—	1.36	—
10–3	T	8,450	10,500	51.0	3.78	3.09	1.37	1.34
10–3	T	8,450	10,500	70.0	3.78	—	1.40	—
10–11	T	7,750	9,700	—	2.38	2.03	1.32	1.27
10–11	T	8,000	10,000	81.0	2.72	—	1.37	—
Av.		8,463	10,650					

The Coefficient of Variation (CV) for the vitamin A unitage of 11 runs = 1.55.
The CV for False Recovery = 18.5.
Actual Check Recoveries for 2 runs when added to melted margarine prior to saponification = 79% and 80%.

in which the vitamin A can be properly controlled and from which the vitamin is eluted in from 5 to 10 minutes are of the correct speed and activity and give the most satisfactory results. In our routine analytical work, only columns that meet these conditions are used.

The next table, Table 4, while not a collaborator's work, gives results obtained in the Associate Referee's laboratory, and are given to indicate the reproducibility and general performance possible by the use of the 1946 amended method, on the 1945 T sample with which practically all of the 1945 collaborators had the most difficulty.

Through all 11 determinations the reproducibility of unitage values was

indicated by the CV of only 1.55 which is probably as good as could be obtained with any existing method. By adding standard to a part of the margarine before the addition of alcohol, reproducible recoveries of 79 per cent and 80 per cent, respectively, were obtained.

The most irregular results on vitamin A unitage value in the 1946 study were reported by Collaborator 4. This was pointed out in Table 3. Nevertheless it was evident that he had done a lot of good work, since he provided complete data on 4 determinations of vitamin A in margarine, with 4 accompanying recovery determinations. His results are presented in more detail in Table 5. The comparisons of the recovery percentages

TABLE 5.—*Table showing abnormal and normal assay recovery and absorption ratio values*

	SAMPLE	TYPE OF ANALYSIS	UNITS VIT. A PER LB. FALSE	RECOV. %	D 290/340	D 325/340
	J	Regular	3,130		*8.19*	1.33
	J	Recovery		*65.6%*	5.64	1.32
	J	Regular	6,400		*5.88*	1.43
Collaborators'	J	Recovery		*174%*	2.33	1.37
Abnormal Relationships	J	Regular	7,900		*5.14*	1.32
	J	Recovery		*156%*	2.65	1.37
	J	Regular	13,000		3.77	1.38
	J	Recovery		*61%*	4.10	1.41
	T	Regular	8,800		3.14	1.37
	T	Recovery		*92.5%*	2.50	1.34
	T	Regular	9,050		3.51	1.37
Laboratory F&D	T	Recovery		*60%*	2.76	1.34
Normal Relationships	T	Regular	8,450		3.78	1.37
	T	Recovery		*70%*	3.09	1.34
	T	Regular	8,000		2.72	1.37
	T	Recovery		*81%*	2.03	1.27

with corresponding $D\frac{290}{340}$ ratios are believed to point directly to the fundamental cause of most of the collaborators' difficulties.

In the first place, this collaborator because of some unrevealed peculiarity in technique or materials, obtained excessively low values in the regular runs as well as in the initial recovery of the vitamin A standard. This with the accompanying excessively high $D\frac{290}{340}$ ratios simply means that the vitamin A was destroyed. However, the most startling and revealing point contained in his data is that apparent recoveries of 156 per cent and 174 per cent were obtained with greatly lowered and more nearly normal $D\frac{290}{340}$ ratios. Practically the only meaning of such a phenomenon in the light of past experience is that the excessive deterioration occurred by virtue of the improper conditions imposed which, however, were almost

completely neutralized by something accompanying the recovery standard. It is believed that this "something" probably is in the nature of a stabilizer or antioxidant. If this analysis is correct it gives an approach to a definite improvement of the method.

This belief was supported by subsequent correspondence with this collaborator, revealing that he had used a saponified standard solution for the initial standard recovery trial, but that he had used a whole distilled ester solution added to the melted margarine sample before the addition of alcohol or the starting of the saponification for the recovery trial with margarine.

The hitherto unexplainable low and generally irregular recoveries of vitamin A standard when added to the alcoholic margarine samples in the "chrom-spec" procedure are believed to further illustrate stabilizer relationships. The vitamin A originally present in the margarine is believed to

TABLE 6.—*Procedure losses of vitamin A as based on absorption at 325 mμ.*

	PER CENT
1. Loss due to chromatographing alone	2.0
2. Loss due to heat evaporation alone	22.0
3. Loss due to heat evaporation plus chromatographing	65.0
4. Loss due to saponification and chromatographic concentration and chromatographic separation plus 10 drops cottonseed oil	17.0

be stabilized because of substances present in the margarine but not in the standard when it is added just prior to the saponification. The ever-present peak around 290 mμ–295 mμ indicates that a tocopherol may be pertinent to this matter. At any rate, the standard could be largely "flash" destroyed upon its addition because of its own insufficient stabilization. A study of stabilization relationships should result in an effective decrease or elimination of this sort of discrepancy. Some experiments performed last summer in connection with other vitamin A studies seem to throw further light on this matter. Such results are given in Table 6.

It has been the practice in this laboratory to hold previously prepared vitamin A standard solutions until the spectrophotometric examination indicates a significant drop in the 325 mμ absorption. Solutions kept in brown bottles over sodium sulphite generally keep very well according to this criterion for at least three months.

Such a vitamin A alcohol standard in petroleum ether is the material referred to in Table 6. This material when chromatographed alone lost only 2 per cent. Evaporating to dryness alone caused a loss of 22 per cent

but chromatographing alone after this heating resulted in an over-all loss of 65 per cent. In another run, where the standard was put through a margarine saponification procedure, a chromatographic concentration, and a chromatographic separation with a stabilizer composed of 10 drops of cottonseed oil, the over-all loss was only 17 per cent.

These values are regarded as neither absolute nor ultimate figures to be expected by controlled stabilization in the vitamin A in margarine technique, but they do indicate the nature of what can be reasonably expected by some recognition and control over stabilization factors.

During the past few years the antimony trichloride method has come into rather general use as a plant control method for margarine. The reproducibility figures further support some adherence to the method. The Associate Referee recognizes the worth of the method in some respects but still it does have very serious and fundamental limitations, which are seldom mentioned. It was with some of these limitations in mind that the work with chromatographic method was originally undertaken. Other limitations and facts relative to the antimony trichloride method became even more apparent during the course of the work. Some of these, may be enumerated, with the reasons, as follows:

(1) Only those who have access to the materials used in margarine manufacture can hope to make a reasonable blank correction with the antimony trichloride method.

(2) Antimony trichloride–vitamin A relationships are affected by the nature of the margarine, probably more particularly by the stabilizers or antioxidents still present. In experimental work unexplainable low values are sometimes obtained. Furthermore, samples reported low by other laboratories using the antimony trichloride method, have been referred to us and not found low by biological assay or by the "chrom-spec-photo" method.

(3) The increment antimony trichloride method cannot correct for the blank with only the finished product available. Also, it is without proof that the vitamin A added as a standard will always behave in the same manner as with the differently treated or associated vitamin A already present.

(4) Both red and green colors which develop rapidly enough to interfere with the antimony trichloride test have been observed in some margarine samples. Some colored samples are particularly troublesome in this respect.

In the collaborative studies on margarine, it had been indicated that two principal factors are responsible for the variability found in different laboratories, and it had been shown that one of these factors, the stability of vitamin A during the manipulations, is a most important consideration. Furthermore, completed work indicates that the addition of stabilizers during the procedure would greatly improve the recoveries and the reproducibility of the vitamin A determinations. However, it should be remembered that stabilizers vary greatly in character and interrelationships in biological materials, showing that such stabilizers may only be effective at critical concentrations and in definite ratios with each other.

The other important factor effecting variation of results between laboratories is the speed and activity of the column. Successful continued use of the "chrom-spec-photo" method (without added stabilizers) has demonstrated that the column of the proper speed and activity is the one in which the vitamin not only can be controlled, but one which can be eluted within 5 to 10 minutes.

With more information about the proper use of stabilizers in this procedure, the speed and activity of the column may be less critical.

Further independent investigation, as well as collaborative work, should be conducted with the intent of maintaining improved stabilization in all vitamin A manipulations.

Even though some workers have success with the method "as is," it appears that the work should be continued along the suggested lines to its indicated more successful conclusion from the more extensive collaborative viewpoint.

Collaborators actively furnishing data for this study are as follows:

LABORATORIES COLLABORATING

(1) University of Southern California, Dr. H. J. Deuel, Jr.
(2) The Fleishmann Laboratories, 810 Grand Concourse, New York 51, N. Y. (Harold K. Steele).
(4) Best Foods, Inc., Laboratory, 99 Avenue A, Bayonne, N. J. (H. W. Volteich, R. H. Neal, and Frederick H. Luckmann).
(5) Distillation Products Inc., Rochester 13, N. Y. (Edgar M. Shantz, Norris Embree).
(7) Army Service Forces, QMC Subsistence and Development Laboratory (Sadie Brenner).
(9) John F. Jelke Co., 759 So. Washtenaw Ave., Chicago, Ill.
(11) Kraft Cheese Co., 500 Peshtigo Court, Chicago, Ill.
(18) Swift and Co., Chicago, Ill. (Dr. E. E. Rice).
(19) National Oil Products Co., Research and Control Laboratories, Harrison, N. J. (Kenneth Morgareidge, Director).

No reports were given on vitamin B^1, vitamin C, vitamin D–milk, or vitamin D–poultry.

REPORT ON RIBOFLAVIN (MICROBIOLOGICAL)

By A. R. KEMMERER (Department of Nutrition, Agricultural Experiment Station, University of Arizona, Tucson),
Associate Referee

The report for 1946 is the same as for 1945 (*This Journal*, **29**: 25). Since the recommendations were not acted upon at that time they are given herewith.

RECOMMENDATIONS*

It is recommended—

(1) That the microbiological method for the determination of riboflavin described in *This Journal*, **29**: 25, 1946,† be adopted as tentative and that the methods previously adopted be deleted (*This Journal*, **24**: 413; **25**: 459; **27**: 540).

(2) That collaborative work on the bacteriological method for riboflavin be discontinued until there is further need for it.

REPORT ON CHEMICAL METHODS FOR RIBOFLAVIN

By HENRY W. LOY, JR. (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Two different types of assays for riboflavin were described in 1939. The work of Snell and Strong (1) on a microbiological procedure for the determination of riboflavin served to introduce the now widely used practice of assay of nutritive factors by means of growth response of suitable microorganisms. The microbiological assay for riboflavin, in a form identical in all essential details with that described in the U.S. Pharmacopoeia (2), was studied by this Association (3).[1]

Also in that year Hodson and Norris (4) published a procedure for the determination of riboflavin based upon a measure of its fluorescence in aqueous solution. They used an indirect method because of the usual presence of interfering fluorescent substances and pigments that accompany riboflavin upon its extraction from foodstuffs. In the direct method, any interfering fluorescence in the green portion of the spectrum will cause a high result. Interfering pigments absorb some of the activating and fluorescent light and will cause a low result. Also, certain electrolytes have a quenching effect upon the riboflavin fluorescence and will cause a low result. It was found for the samples examined that a 2-step reduction would eliminate the effect of these interfering substances.

The indirect method of Hodson and Norris is based upon the following properties of riboflavin: (*a*) its solution when irradiated with blue light fluoresces green; (*b*) it withstands mild oxidation or reduction; (*c*) it is reduced to a nonfluorescing form with sodium hydrosulfite, but is easily reoxidized to the fluorescing form by shaking with air; and (*d*) it is not reduced with stannous chloride. A preliminary sodium hydrosulfite-stannous chloride reduction lessens or eliminates the interference of other fluorescent substances and pigments. In the presence of stannous chloride, riboflavin may be reoxidized to the fluorescent form while most other

* For report of Subcommittee A and action by the Association, see *This Journal*, **30**: 44 (1947).
† *See also*, under "Changes in Methods," *This Journal*, **30**: 79 (1947).
[1] The method was adopted as tentative, *This Journal*, **30**, 79 (1947).

fluorescent substances remain reduced so that they do not interfere. Furthermore, undesirable absorption of activating and fluorescent light by interfering pigments may be decreased by this latter step.

In this method sodium hydrosulfite solution and stannous chloride solution are first added to the sample solution. After mixing and standing the riboflavin is reoxidized by shaking with an excess of air. The fluorescence of the solution is measured, then a standard riboflavin solution of known fluorescence is added and the fluorescence is measured again. The extent to which any interfering pigments decrease the measurement of riboflavin is determined in this manner, and the amount of fluorescence due to the sample solution is calculated. The riboflavin is then reduced with sodium hydrosulfite and the fluorescence of the solution is measured again to determine the effect of foreign stable substances that fluoresce green. The amount of riboflavin in the sample is then calculated.

This method has been studied and modified by a number of workers (5) to (17) and has had wide use, particularly for purposes of plant control. The method, with slight modification, was studied collaboratively by this Association (5) to (7) in 1941–2 and in 1943, and was adopted as a tentative method for the assay of yeast, dried skim milk, and alfalfa (18) (19). This method has since been dropped as it was found to be unsatisfactory for many products.

During this period the Hodson-Norris method, in its various modified forms, was studied by a number of workers who failed to obtain results with it that were in agreement with values obtained by microbiological assay procedures. Najjar (20), in 1941, for the assay of riboflavin in blood and urine, proposed the use of pyridine-butyl alcohol extraction of the riboflavin, to avoid reading fluorescence of turbid aqueous solutions, as well as interference from gaseous emulsions that persist in aqueous solutions. Also, many interfering pigments that may not be destroyed by permanganate treatment are soluble in water, but not in butyl alcohol. This method appeared to have a decided advantage, and in an extensive study in our laboratory it was modified in attempts to apply it to pharmaceutical and food products. Najjar, in working with urine, found that simple oxidation with permanganate was sufficient to inactivate the non-riboflavin fluorescing substances. The excess permanganate was then removed with peroxide. Separation of riboflavin from the aqueous medium was accomplished by addition of pyridine, with subsequent saturation of the mixture with anhydrous sodium sulfate. This pyridine then separated as a surface layer and contained most of the riboflavin present. Addition of butyl alcohol completed the extraction of the riboflavin, and fluorescence of the pyridine-butyl alcohol layer was measured.

In applying this procedure to many commercial products containing riboflavin it was found possible to dispense with the pyridine. Samples were extracted by autoclaving from 30 to 60 minutes in tenth-normal

hydrochloric acid, then carried through the precipitation procedure recommended by Loy and Kline (21) for preparation of sample solutions for microbiological assay. After proper dilution the sample solution was treated with acetic acid, permanganate, peroxide, extracted with butyl alcohol, and the fluorescence was compared with that of a riboflavin standard solution treated in the same manner. That this procedure was satisfactory for most pharmaceutical preparations containing riboflavin was indicated by comparison of values obtained with those of microbiological assay. There was excellent agreement for the results of the various capsule and tablet preparations, to which riboflavin was undoubtedly added in crystalline form. An appreciable discrepancy was found, however, in results with dried brewers yeast. Difficulty in obtaining good agreement between these two methods was further experienced in the assay of some flour and bread samples. Such samples were further extracted with pyridine, digested with various enzymes, or extracted by autoclaving for longer periods of time, without obtaining the total riboflavin present in a form that could be measured in butyl alcohol. These results suggest that riboflavin may be present in natural sources in a bound form that is water-soluble, but is insoluble in butyl alcohol. By analogy with free thiamine-thiamine pyrophosphate relationship, and the solubilities of the resulting thiochrome in water and isobutyl alcohol, it is suggested that riboflavin may occur naturally as a phosphate that requires for its splitting an enzyme not present in the clarase, mylase, polidase, pepsin, or trypsin preparations that have been used. It was clear from these results that the butyl alcohol extraction procedure is unsuitable as a general procedure for control purposes.

The American Association of Cereal Chemists (13) in 1944–45 conducted a collaborative study of a fluorometric method that employed oxidation of the sample solution with permanganate, similar to that used by Najjar. Determination was then made of the fluorescence of the sample solution, the sample solution with added riboflavin standard, and again after reduction with sodium hydrosulfite. Acceptable results were obtained by a number of collaborators who assayed samples of partially enriched flour and bread.

The Hodson and Norris method was further studied critically by Rubin, De Ritter, Schuman, and Bauersfeind (16) and so modified that good agreement with the microbiological procedure (1), (22), (23) was obtained. Many of the riboflavin methods have been found satisfactory when applied to the types of products for which they were designed. This laboratory had difficulty, however, in their application as general control procedures to many types of products of unknown composition. The criteria for acceptability include not only ease of handling, rapidity, availability of apparatus, and reproducibility, but satisfactory comparison with the microbiological procedure which is regarded as reliable from the standpoint of accuracy as well as specificity.

During the past year considerable time and effort has been spent in the study of a method in which are combined, it is believed, the best features of those previously described, particularly those of Najjar (20), Arnold (13) and Rubin *et al.* (16).

In this procedure, extraction of the sample with 0.1 *N* hydrochloric acid instead of 0.1 *N* sulfuric acid is used. Some question has arisen in

TABLE 1.—*Comparison of microbiological and modified fluorometric procedures for riboflavin in various products*

PRODUCT		METHOD	
		MICRO-BIOLOGICAL	MODIFIED FLUOROMETRIC
	Per	*Mg. of Riboflavin*	
1. Enriched flour	lb.	1.90	1.82
2. " "	"	1.12	1.06
3. " "	"	1.38	1.44
4. Enriched bread (fresh basis)	"	0.92	0.94
5. " " " "	"	0.95	0.97
6. " " " "	"	0.93	0.94
7. Dried brewers yeast	g.	0.050	0.048
8. " " "	tab.	0.040	0.041
9. " " "	"	0.052	0.050
10. Fresh bakers yeast	g.	0.021	0.021
11. Liver extract powder	"	0.34	0.34
12. Linseed oil meal	"	0.0028	0.0027
13. Alfalfa leaf meal	"	0.012	0.011
14. Dried skim milk	"	0.022	0.019
15. Vitamin B complex	tab.	0.18	0.19
16. Brewers yeast with B complex	"	1.99	2.00
17. Dietary supplement	"	2.40	2.38
18. Concentrated vegetable broth powder	av. oz.	0.40	0.40
19. Fortified liver and iron solution	ml.	0.73	0.72
20. Yeast concentrate with multivitamins	tab.	0.47	0.47

this laboratory of the possibility of the adsorption of a measurable amount of riboflavin from the sample solution, in the assay of certain types of products, when an appreciable amount of sulfate precipitates are formed upon neutralization or partial neutralization of the sample solution. The sample mixture is autoclaved for 30 minutes. It was thought that more accurate results might be obtained by longer autoclaving, for certain types of materials, and, also, that the same sample solution is suitable, when needed, for the microbiological assay.

After autoclaving and cooling, the mixture is brought to a *p*H of approximately 6.0 and carried through the precipitation procedure recommended by Loy and Kline (21). After the sample solution has been prepared, all reactions are carried out in the fluorometer tubes, thus saving manipulations with additional glassware. Oxidation of the sample solution with potassium permanganate is carried out at a lower *p*H than

that recommended by Arnold (13), and the time of oxidation is limited, making the recovery step of Rubin *et al.* (16) unnecessary.

Results of a number of assays of various types of samples are compared with microbiological results in Table 1. These were selected at random and are typical of the agreement that has been obtained with these two methods. The greatest variation between the two methods is less than $\pm 5\%$ except for low-potency materials.

From the work so far carried out in this laboratory it appears that the method described will meet the needs of control officials as well as of those interested in plant control.

RECOMMENDATION*

It is recommended that the fluorometric method for the assay of riboflavin herein described be studied collaboratively during the coming year.

The complete text of the method follows:

ASSAY PROCEDURE

REAGENTS

(a) *Standard riboflavin stock soln.*—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard in sufficient 0.02 *N* acetic acid soln to make 500 ml. Store, protected from light, under toluene at ca 10°. 1 ml = 100 micrograms of riboflavin.

(b) *Standard riboflavin soln.*—Dilute 1 ml of (a) with H_2O to make 100 ml. 1 ml = 1 microgram of riboflavin. Prepare fresh standard soln for each assay.

(c) 10 *N HCl soln.*

(d) 1 *N HCl soln.*

(e) 0.1 *N HCl soln.*

(f) 10 *N NaOH soln.*

(g) 1 *N NaOH soln.*

(h) 0.1 *N NaOH soln.*

(i) *Glacial acetic acid.*

(j) 4% $KMnO_4$ *soln.*

(k) 3% H_2O_2 *soln.*

(l) $Na_2S_2O_4$ *powder.*

APPARATUS

Electronic photofluorometer.—Use a fluorometer that is suitable for accurately measuring fluorescence of solutions containing riboflavin in concentrations of ca 0.1 to 0.2 microgram per ml.

PREPARATION OF SAMPLE SOLUTION

Throughout all stages of the procedure, protect the soln from light that destroys riboflavin.

The quantity of material taken for assay depends upon amount available and riboflavin content. Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*—Add a volume of 0.1 *N* HCl soln equal in ml to at least ten times the dry weight of the sample in g, but the resulting soln shall contain not more than 100

* For report of Subcommittee A and action by the Association, see *This Journal*, 30: 44 (1947).

micrograms of riboflavin per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, shake vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 0.1 *N* HCl soln.

Autoclave the mixture at ca 121° (1 kg per sq cm) for 30 min. and cool to room temperature. Adjust the mixture to ca *p*H 6 with NaOH soln[1] and add HCl soln immediately until no further precipitation occurs (usually ca *p*H 4.5, the isoelectric point of many of the proteins). Dilute the mixture to a measured volume that contains more than 0.1 microgram of riboflavin per ml and filter through paper known not to adsorb riboflavin. In the case of a mixture that is difficult to filter, centrifuging and/or filtering through sintered glass (using a suitable analytical filter-aid) may often be substituted for, or may precede, filtering through paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then NaOH soln, and proceed as follows:

(1). If no further precipitation occurs, add NaOH soln to a *p*H of 6.6–6.8, dilute the soln to a final volume that contains ca 0.1 microgram of riboflavin per ml., and if cloudiness occurs, filter again.

(2). If further precipitation occurs, adjust the soln again to the point of maximum precipitation, dilute to a volume that contains more than 0.1 microgram of riboflavin per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

If the riboflavin content of the sample is too low so that these requirements cannot be met, then concentrate the clear filtrate obtained at ca *p*H 4.5 to a suitable volume with heat under reduced pressure. Filter if necessary and then proceed as outlined above.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add HCl soln and adjust the mixture to ca *p*H 6. Add such an amount of H_2O that the total volume of liquid shall be equal in ml to not less than ten times the dry weight of the sample in g. Then add the equivalent to 1 ml of 10 *N* HCl soln for each 100 ml of liquid and proceed as directed under **(a)**.

(c) *For liquid materials.*—Adjust the material to ca *p*H 6 with either HCl soln or NaOH soln and proceed as directed under **(b)**.

DETERMINATION

To each of 4 or more tubes[2] (or reaction vessels) add 10 ml of sample soln. To each of 2 or more of these tubes add 1 ml of the standard riboflavin soln, and to each of 2 or more of the remaining tubes, add 1 ml of H_2O. To each of the tubes add 1 ml of glacial acetic acid, mix, add 0.5 ml of $KMnO_4$ soln,[3] mix immediately, and allow to stand for 2 min.[4] Then to each of the tubes, add 0.5 ml of H_2O_2 soln, and mix immediately, whereupon the permanganate color must be destroyed within a few seconds.[5] (A measured volume of acetone, with mixing, or one drop of caprylic alcohol, may be added to each tube if frothing occurs.)

In a suitable fluorometer, measure the fluorescence of the sample soln with the added standard riboflavin and call this reading A. Next, measure the fluorescence of

[1] The concentrations of the HCl and the NaOH solns are not stated in each instance because these concentrations may be varied depending upon the amount of sample taken for assay, volume of sample solution, and buffering effect of sample.

[2] If the fluorometer is of a type that requires tubular cuvettes, all reactions may be carried out in a matched set of these cuvettes.

[3] The amount of $KMnO_4$ soln may be increased for sample solutions that contain an excess of oxidizable material, but do not add more than 0.5 ml in excess of that required to complete the oxidation of foreign material.

[4] The Associate Referee has not found that it was necessary to include a riboflavin recovery step in this procedure, as no destruction of riboflavin has been observed until an elapse of time of ca 3 minutes.

[5] In the samples assayed in this laboratory with the precipitation procedure properly followed for preparation of sample soln, no precipitate has formed during the above reactions and, therefore, filtration at this point was not necessary.

the sample soln containing 1 ml of added H_2O and call this reading B. Then add 20 mg of $Na_2S_2O_4$,[6] to reduce the riboflavin present, to one or more tubes of reading A and one or more tubes of reading B, mix contents of each tube, measure the fluorescence immediately, and call this reading C.[7] The riboflavin content may then be calculated as follows:[8]

$$\text{Mg of riboflavin per ml of final sample soln} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}.$$

Calculate the amount of riboflavin in the sample on the basis of the aliquots taken during the analysis.

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REPORT ON NICOTINIC ACID

By F. M. Strong (Department of Biochemistry, University of Wisconsin, Madison, Wis.), *Associate Referee*

The discovery of the nutritional significance of nicotinic acid (1) made imperative the development of methods for the determination of this substance in biological products. Although various chemical and biological

[6] The $Na_2S_2O_4$ must be of high purity. Do not use if unduly exposed to light or air. An amount appreciably in excess of 20 mg may reduce foreign pigments and/or foreign fluorescing substances and cause erroneous results.

[7] After reduction, tubes of A and B should give the same degree of fluorescence.

[8] It is believed that most accurate results are obtained when the sample soln is of such a dilution that B–C/A–B approaches 1/1. Any determination in which there is an appreciable deviation from this ratio, would be considered a preliminary assay and should be repeated using a dilution of the sample soln that would give readings more nearly approaching this ratio.

methods have been proposed, the preferred procedure at the present time appears to be the microbiological assay of Snell and Wright (2). A full account of the development, use, and recent modifications of this method has recently been written by Snell (3).

The microbiological assay was adopted by the United States Pharmacopoeia after collaborative study on samples of a pharmaceutical nature, and the U.S.P. version of the method was tentatively accepted by the Association of Official Agricultural Chemists in 1943 (4). However, no collaborative study under A.O.A.C. auspices was undertaken at that time. Subsequent improvements in the procedure and the need for testing its efficiency when applied to agricultural products indicated the desirability of such work, which was started in 1944.

For this purpose detailed directions for the Snell-Wright assay, incorporating as many as possible of the recent improvements, were drawn up, and the method was submitted to collaborative study. The samples assayed were: (*1*) dried, defatted pork muscle, (*2*) skim milk powder, and (*3*) whole wheat flour. A pure sample of nicotinic acid was also sent each collaborator. The details of the method used differed from the tentative A.O.A.C. method in several respects. The concentrations of tryptophane, pyridoxine, and biotin in the basal medium were halved, while those of glucose and sodium acetate were increased to 2 per cent each. The reasons for these changes were that the first three ingredients appeared to be present in the basal medium in an unnecessarily large excess over the requirements of the test organism, whereas the increased amounts of glucose and sodium acetate resulted in a more desirable type of standard curve (5).

The method of handling cultures and preparing inoculum was also changed somewhat. The glucose in the agar medium for stab cultures was reduced from 1 to 0.5 per cent, and it was directed that cultures more than 1 week old be discarded. This was designed to minimize exposure of the bacteria to lactic acid. It was also specified that inoculum be prepared directly from a stab culture rather than from previous inocula. The liquid medium in which the inoculum is grown probably does not meet the growth requirements of *Lactobacillus arabinosus* as well as the rich, natural, agar medium. Inocula from the stab should, therefore, be more vigorous. The practice of centrifuging the inoculum and resuspending the cells in saline solution was discontinued, since satisfactorily low blanks could be obtained without this precaution. The numerous other changes were too minor to warrant individual comment.

COLLABORATORS

The following collaborators took part in this study. The author wishes to extend his sincere thanks to them for their generous cooperation:

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SUMMARY OF COMMENTS OF COLLABORATORS

Esther P. Daniel.—Numerous small changes in the U.S.P. method probably make little real improvement and are undesirable because the U.S.P. and A.O.A.C. methods should be identical. However, increasing the glucose and sodium acetate in the basal medium seems to reduce variations in the potency of cereal samples calculated from different levels of the sample solution, and is, therefore, desirable. One per cent sodium acetate is preferable to 2 per cent, because the latter increases the difficulty of securing a sharp end point in the titrations.

The use of unwashed inoculum suspensions and the elimination of serial transfers in the liquid medium for growing inoculum are regarded as distinct improvements. The primary standard used should be the U.S.P. reference standard nicotinic acid. The autoclaving period of 12 minutes at 15 pounds pressure should be raised to 15 minutes.

Owing to the difficulty in obtaining uniform samples of many materials it would be desirable to use larger samples. Sample levels should be selected so as to utilize as much of the linear part of the standard curve as possible.

R. A. Stewart.—On two occasions it was found that 12 minutes autoclaving at 15 pounds was not sufficient time to sterilize all the tubes. The 15 minute procedure was adopted thereafter.

Sadie Brenner.—It was found preferable to take larger samples than 0.1 to 1.0 gram as recommended, and make more dilutions.

K. George Falk.—Niacin standard was found to be more stable in alcohol (absolute ethyl) than in an aqueous solution. Collaborative media inoculated with unwashed culture gave a higher blank than U.S.P. media inoculated with washed culture. Titrations were carried out directly in the tubes with air stirring.

P. B. Pearson.—In the past various methods of treating the sample to liberate nicotinic acid have been tested, and the results after autoclaving with N sodium hydroxide have not been any higher than with 0.1 N hydrochloric acid. It would be desirable further to study the liberation of nicotinic acid by autoclaving with 0.1 N hydrochloric acid.

The standard curves are slightly more consistent when one-half as much sodium acetate as the amount recommended in the A.O.A.C. method is used.

Elsa Orent-Keiles.—Much better agreement between samples is usually obtained when large-sized samples are taken. The amounts of the products sent for collaborative study did not provide sufficient material for adequate sampling.

The increased concentration of sodium acetate exhibits such a marked buffering effect that against 0.1 N sodium hydroxide a sharp end point is usually very difficult to determine.

DISCUSSION OF 1944 RESULTS

The results obtained in the 1944 study are shown in Table 1. Each individual assay was checked to make sure that it conformed to the requirements listed in the collaborative procedure, and all which failed to do so have been excluded from the table. The calculations were repeated whenever there was any question on this point.

TABLE 1.—*Results of 1944 A.O.A.C. collaborative study on nicotinic acid*

ANALYST	SAMPLES[1]		
	1	2	3
		micrograms per gram	
1	248	8.60	52.8
	240	9.45	56.7
	242	9.79	53.2
		9.10	55.9
2	192	6.30	46.5
	203	6.30	45.7
3	238	9.80	48.2
	249		47.0
4	257	8.10	52.9
	249	9.03	52.5
	245	9.25	51.0
	256	8.20	52.4

TABLE 1.—(*continued*)

ANALYST	SAMPLES[1]		
	1	2	3
		micrograms per gram	
5	247	7.69	44.2
	235	7.38	42.5
	227	7.66	44.5
6	233	9.73	57.0
	239	8.66	47.5
	240		
7	240	8.54	48.4
	244	8.54	48.2
	242	8.05	51.0
	243	8.51	52.2
	237	8.01	47.6
	250		51.3
	241		46.2
8	232	8.18	50.5
9	266	7.91	53.0
	238	7.54	57.2
	235	7.56	55.5
10	257	8.90	41.1
	256	9.04	44.7
	224	9.56	45.1
		9.40	42.8
11	224	8.96	45.8
	247	9.64	55.2
	241	7.94	
12	222	7.95	51.4
13	272	9.50	76.8
	241		
14	240	8.84	53.2
	243	8.90	52.5
	243	8.76	52.4
	239	8.88	46.4
	269	8.84	55.4
	245	8.70	56.2
15	255	10.1	48.2
	250	9.72	54.8

TABLE 1.—(*continued*)

ANALYST	SAMPLES[1]		
	1	2	3
		micrograms per gram	
16	240	8.40	51.1
	226	8.22	53.5
	247		
17	260	8.29	52.5
	248	8.33	52.5
	253	8.57	52.3
		8.77	
		9.40	
18	231	7.93	46.5
	233	7.80	42.9
19	245	9.55	52.6
	248		56.5
	261		57.5
20	264	9.60	70.5
	256	9.40	64.6
21	240	8.86	44.0
	216	8.14	48.3
		8.86	47.8
		9.04	48.5
		16.7	40.6
		10.6	
22	238	8.00	47.0
Mean	242	8.79 (8.65)[2]	51.0
Std. error of mean	2.7	0.206 (0.182)[2]	1.46
Std. error of mean of 2 determinations by a single collaborator	12.3	1.017 (0.778)[2]	6.13

[1] Sample 1—dried, defatted pork muscle; Sample 2—skim milk powder; Sample 3—whole wheat flour.
[2] Omitting the value 16.7 submitted by collaborator 21.

Inspection of the results in Table 1 reveals a very satisfactory degree of agreement among the values secured by the various collaborators. The most significant value is probably that for the standard error of the mean of two determinations by a single collaborator selected at random

(last figure in the table). These values amount to 5.1, 11.6 and 12.0 per cent of the means for samples 1, 2, and 3, respectively.

Although the 1944 study showed quite clearly that the microbiological method for nicotinic acid was capable of yielding results of acceptable precision, several considerations pointed to the desirability of further work. These were the need for a uniform procedure for both the U.S.P. and the A.O.A.C. methods, the observation that the standard curves obtained by a majority of the collaborators were very low (less than 6 ml. of 0.1 *N* acid production at 0.3 mmg of nicotinic acid) while others were very good, and finally the possibility of simplifying the method so as to reduce materially the time required.

1945 U.S.P.-A.O.A.C. COLLABORATIVE ASSAY METHOD FOR NICOTINIC ACID

Accordingly, in 1945 a collaborative study sponsored jointly by the U.S.P. and the A.O.A.C. was carried out. Five samples were assayed: (1) whole wheat flour, (2) dried skim milk, (3) dried yeast, (4) a liquid vitamin B complex concentrate, and (5) a recovery sample consisting of the B complex concentrate containing 520 mmg of added nicotinic acid per gram. Sample 1 was identical with sample 3 of the 1944 study. Each collaborator was also furnished with a vial of U.S.P. nicotinic acid reference standard to be used as the primary standard, and with a culture of the test organism, *Lactobacillus arabinosus 17–5*. The collaborators were asked to transfer the test organism in stab culture at least twice, at one or two day intervals, before using it for assay work.

The details of the method are given in *This Journal*, **30**: 82, under "Changes in Methods"; Table 2 gives detailed results by each analyst.

Larger amounts of the test sample are used than in the 1944 work, the sodium acetate content of the basal medium is reduced to 1 per cent, and the time of autoclaving the tubes is increased to 15 minutes. Likewise several of the stock solutions have been combined and their concentrations have been so adjusted that only two different volumes need to be measured out. The method of calculating the results has also been altered slightly.

COLLABORATORS, 1945

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Elmer B. Vliet, Abbott Laboratories, North Chicago. Ill.
Sadie Brenner, Army Service Forces, Q.M.C. Subsistence Laboratory, Chicago 9, Ill.
John A. Buchter, Lakeside Laboratories, Milwaukee 1, Wis.
Elsa Orent-Keiles, Agricultural Research Administration, Bureau of Home Economics, Washington 25, D. C.

SUMMARY OF COMMENTS OF COLLABORATORS

Esther P. Daniel.—The convenience and simplification afforded by combining the vitamin solutions and adjusting volumes is a desirable change. Using the inoculum without centrifuging saves time and is just as satisfactory as the former procedure. Several daily transfers of a new culture before use and at least one transfer per week thereafter is recommended. In some cases 10 or 12 transfers have been required for best results with a new culture.

Louise J. Daniel.—The method as used is relatively short and quite satisfactory. It might be advisable to filter the medium and sample before placing the aliquots in the assay tubes, since some samples might cause stimulation.

Lawrence Atkin.—The combined stock solutions are very convenient to use. The hydrolyzed casein solution did not show any precipitate, due probably to the low *p*H and this is an improvement. The simplified procedure for preparation of the inoculum gives satisfactory results without the production of an unduly high blank.

K. George Falk.—Collaborative media inoculated with unwashed culture gave a higher blank than U.S.P. media inoculated with washed culture.

Rosa Abbott.—Unless the procedure is simplified it is too cumbersome for control purposes.

Sereck H. Fox.—A large precipitate formed in the acid hydrolyzed casein solution after setting in the refrigerator (4° C.) overnight. This solution was used containing the precipitate and also after filtering off the precipitate with no noticeable change in the results. Consequently, the entire solution was filtered and the filtrate used as the casein hydrolysate in all of the assays reported.

Sterilization of the assay tubes in the manner recommended seemed to cause caramelization of the glucose and erratic results. After diluting to 0.1 microgram per ml it was found necessary to filter the test solution for uniform results.

DISCUSSION OF 1945 RESULTS

The results obtained in the 1945 study are collected in Table 2. Each assay was individually checked and only those meeting all the requirements of the method as detailed above have been included. The agreement among the various assay results has again been expressed in terms of the standard error of the mean and the standard error of the mean of two determinations by a single collaborator selected at random. The latter values amount to 12.9, 11.9, 9.4, 7.3, and 7.1 per cent, respectively, of the means for samples 1 to 5. The recovery of the nicotinic acid added to sample 5, as calculated from the mean values for samples 4 and 5 in Table 2, was 101.7 per cent. It is interesting to note that both the amount of nicotinic acid found and the degree of variation among the individual results observed on the wheat flour sample were closely similar in the 1944 and 1945 studies.

TABLE 2.—*Results of 1945 A.O.A.C.-U.S.P. collaborative study on nicotinic acid*

ANALYST	SAMPLES[1]				
	1	2	3	4	5
			micrograms per gram		
1	52.5	9.51	616	811	1320
	53.0	9.32	585	774	1290
	51.0	8.96	612	792	1260
	52.5		625	781	1300
			600	790	1280
2	56.7	9.65	612	806	1300
	50.1	8.91	597	782	1340
	57.3	10.30	646	837	1330
	55.8	9.47	632	776	1330
	53.4	9.02	608	785	1310
3	42.3	8.24	548	715	1190
	44.8	8.21	531	702	1300
	41.6	7.88		715	1150
4	51.1	7.70	551	813	1430
	50.7		479	820	1480
	50.1				1470
5	50.3	8.30	570	810	1340
	52.5	7.95	615	760	1240
	52.3	8.80	588	810	1410
6	53.0	8.90	570	855	1500
	55.0	10.40	629	715	1130
	51.0	10.10		825	1420
		8.90		828	1320

TABLE 2.—(*continued*)

ANALYST	SAMPLES[1]				
	1	2	3	4	5
			micrograms per gram		
7	47.2	9.06	605	994	1400
	41.3	8.89	635	928	1240
	37.5	9.59		917	1320
8	35.8	8.58	601	737	1260
	36.2	8.41	627	769	1240
	39.4	8.04	601	803	1270
	42.4		590	813	1340
9	56.8	12.30	658		
	63.3	12.50	758		
	56.7	10.50	580		
10	45.4	8.11	608	746	1200
	46.9	7.66	557	701	1200
	45.1	7.77	564		1350
11	66.0	9.35	715	923	1480
12	51.0	8.20	543	800	1280
	49.0	8.70	560	790	1260
	51.0	9.10		780	1270
				780	
13	42.5	9.39	571	741	1310
	47.2	8.60	560	820	1380
14	51.1	12.10	611	801	1220
	56.0	12.60	663	806	1370
	56.0	11.20	655	869	1280
	57.1	11.10		803	1440
	51.5			846	1320
15	40.6	7.00	455	693	1190
	43.9	8.30	459	717	1200
	41.2	7.91	468	663	1110
16					1590
17	53.9	9.35	543	821	1370
	50.1	8.25	689	802	1330
	55.7	9.81	543	863	1320
	55.6			807	1400
18	41.0	8.20	505	837	1440
	41.8	7.70	473	713	1570
	31.0	8.00	426	628	1190

TABLE 2.—(*continued*)

ANALYST	SAMPLES[1]				
	1	2	3	4	5
			micrograms per gram		
19	51.9	8.55	560	779	1310
	55.3	8.84	590	785	1350
	54.5	10.00	620	833	1380
	52.4	8.85	598	797	1380
		8.97			
		8.64			
20	51.1	9.10	610	843	1340
	49.7	8.71	592	798	1270
	49.7	8.65	589	775	1290
21	54.2	8.66	623	859	1420
	59.6	8.33	615	845	1470
	56.8	8.57		873	1410
	52.9	8.55		803	1430
		8.50		833	
22	57.5	9.59	660	930	1420
	57.1	10.00		916	1410
	57.7	9.70		892	1420
23	51.2	8.88	625	817	1320
	49.2	9.30	620	790	1400
	49.5	8.70	611	814	1340
			601	815	1240
					1290
					1260
24					
25	45.5	9.10	639	762	1240
	46.0	9.17	632	762	1240
	44.0	8.85		774	1270
	43.8				
Mean	49.9	9.08	591	801	1330
Std. error of mean	1.36	0.232	12.0	12.6	19.5
Std. error of mean of 2 determinations by a single collaborator	6.42	1.097	55.7	58.8	95.1

[1] Sample 1—whole wheat flour; Sample 2—dried skim milk; Sample 3—dried yeast; Sample 4—liquid B complex concentrate; Sample 5—liquid B complex concentrate plus 520 mmg. of added nicotinic acid per gram.

The standard curves obtained during the two years were, however, quite different. For purposes of comparison standard curves were arbitrarily designated as satisfactory if the blank titration was 2.0 ml. of 0.1 *N* alkali or less, and the titration at 0.3 mmg of nicotinic acid was 8.0 ml. or more. Above 0.3 mmg. of nicotinic acid many of the curves were decidedly rounded. On this basis the standard curves reported by 15 collaborators in 1944 were classified as 10 unsatisfactory and 5 satisfactory, while in 1945 only 5 of 25 collaborators had unsatisfactory standard curves. The improvement is probably attributable to alterations in the method of handling the cultures of the test organism, and to the fact that a vigorous culture was sent each collaborator for the 1945 study.

RESULTS OBTAINED BY OTHER METHODS

In both 1944 and 1945 the various collaborators were asked to analyze the samples for nicotinic acid by any other methods which they were using routinely. The results of these analyses as reported by the individual collaborators are collected in Tables 3 and 4. The methods used are all essentially similar either to the Snell-Wright microbiological method (2) or the Dann-Handler chemical method (6). It will be noted that agreement with the A.O.A.C. collaborative results is good in most cases, although the degree of variation in the results for any one sample is often greater than in the collaborative study.

TABLE 3.—*1944 A.O.A.C. collaborative study of nicotinic acid*
Results by other methods

COLLABORATOR	METHOD	SAMPLES[1]		
		1	2	3
		micrograms per gram		
1	U.S.P. XII, except sodium acetate 1%, glucose 2%	247.0	9.2	50.82
		242.0	8.9	50.29
		247.3	9.07	49.86
		250.75	8.9	52.09
3	Melnick, chemical method (7)	244	11.5	51
4	A.O.A.C., enzyme extraction of samples[2]	257.3	15.73	40.4
		257.0	16.10	41.0
	Snell-Wright medium, enzyme extraction	256	15.79	38.15
		254.5	16.20	39.40
	Snell-Wright medium, 1 *N* H_2SO_4 extraction	245	10.1	50.6
		250	9.85	49.7
	Snell-Wright medium, ca 2 *N* HCl extraction	251	9.95	49.13
		254	9.84	51.40

TABLE 3.—(*continued*)

COLLABORATOR	METHOD	SAMPLES[1]		
		1	2	3
		micrograms per gram		
4	Dann-Handler chemical method (6)	250	16.17	50.75
		257	16.00	51.30
		252	17.20	55.30
5	A.O.A.C. medium, turbidity of tubes read after 30 hrs.	250	8.8	50.3
		250	8.3	49.3
6	Dann-Handler[3]	236	12.3	42.8
		223	10.8	41.4
7	Dann-Handler	197	8.34	48.2
		231	8.12	50.5
9	Microbiological[4]	260	8.4	52.1
		235	7.8	53.4
		239	7.9	55.8
		236	7.8	51.6
10	U.S.P. XII	220	9	50
		225	8	50
	Dann-Handler	216		48
		220		50
		224		
11	Microbiological[5]	288	10.9	50.6
16	Snell-Wright[6]	242	10.62	60.1
		245	11.25	59.6
		254	12.30	56.1
		250	11.81	
19	Dann-Handler	230	9.6	52
		229		49
	U.S.P. XII	247	9.5	52
		253		59
22	Snell-Wright	250	8.40	54.3
	Cheldelin *et al.* (8)	236	8.38	50.3

[1] As in Table 1.
[2] Takadiastase and papain, *p*H 4.5, 24 hrs. at 37° C.
[3] Modified by using Lloyd's reagent for purification of the extract, and metol and cyanogen bromide for the color reaction.
[4] Essentially similar to the A.O.A.C. method modified with respect to preparation of casein hydrolysate, medium for and handling of inoculum, and concentration and preservation of stock solutions.
[5] Essentially similar to the A.O.A.C. method modified by addition of asparagine, alanine, and glutamic acid to the basal medium, and autoclaving the samples with *N* NaOH.
[6] Samples digested with takadiastase.

TABLE 4.—*1945 U.S.P.-A.O.A.C. collaborative study of nicotinic acid*
Results by other methods

COLLABORATOR	METHOD	SAMPLES[1]				
		1	2	3	4	5
		micrograms per gram				
1	U.S.P. XII	51	9.1	573	810	1280
		53	9.7	605	790	1280
		53	8.7	600	790	1305
				560	780	1320
					780	1300
6	U.S.P. XII	50	8.34	550	790	1200
	Dann-Handler (6)	51	12	510	860	1200
10	Microbiological[2]	52.2	9.9	577	766	1259
		52.4	10.1	555	780	1270
					806	1300
18	U.S.P. XII	35.4	5.9	445	878	1300
20	U.S.P. XII	47.0	8.29	585	779	1368
23	Chemical	54.9	15.1	490	791	1226
		56.1		518	862	1282

[1] As in Table 2.
[2] Similar to the A.O.A.C. method except that the basal medium contains 1 per cent glucose, and samples are autoclaved with alkali and filtered before assay.

SUMMARY

The microbiological method for determination of nicotinic acid was subjected to collaborative study in 1944 and satisfactory results were obtained. The details of the procedure were subsequently modified to make it essentially identical with the U.S.P. method and to incorporate certain improvements. During 1945 the method was again subjected to collaborative study with very satisfactory results.

RECOMMENDATION*

It is recommended that the 1945 U.S.P.–A.O.A.C. method as described* be made official for the determination of nicotinic acid.

ACKNOWLEDGMENTS

The Associate Referee wishes to acknowledge the fine cooperation of the various collaborators, and to thank Dr. O. L. Kline for assistance in preparing and mailing samples, Dr. E. Fullerton Cook for a supply of U.S.P. nicotinic acid reference standard, and Professor C. Eisenhart for statistical analysis of the results.

* For report of Subcommittee A, and action by the Association see *This Journal*, 30: 44 (1947). The 1945 method is given in detail on p. 82.

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(1) ELVEHJEM, C. A., and TEPLY, L. J., *Chem. Rev.* **33**, 185, (1943).
(2) SNELL, E. E., and WRIGHT, L. D., *J. Biol. Chem.* **139**, 675 (1941).
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(5) KREHL, W. A., STRONG, F. M., and ELVEHJEM, C. A., *Ind. Eng. Chem.. Anal. Ed.*, **15**, 471 (1943).
(6) DANN, W. J. and HANDLER, P. J., *J. Biol. Chem.*, **140**, 201 (1941).
(7) MELNICK, D., *Cereal Chem.*, **19**, 553 (1942).
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REPORT ON CAROTENE (DETERMINATION)

By A. R. KEMMERER (Department of Nutrition, Agricultural Experiment Station, University of Arizona, Tucson),
Associate Referee

The report for 1946 is the same as for 1945 (*This Journal*, **29**:18). Since the recommendations were not acted upon at that time they are herewith given.

RECOMMENDATIONS*

It is recommended.—

(1) That adsorbents be further studied in order to develop tests by which the suitability of an adsorbent for the determination of carotene can be ascertained.

(2) That the methods for carotene previously adopted (*This Journal*, **24**: 859; **25**: 886; **27**: 542; *Methods of Analysis*, *A.O.A.C.*, p. 369, 1940) be dropped and that the method described in *This Journal*, **29**: 18, 1946, for the determination of carotene be adopted as tentative.

No report was given on carotene (chromatic separation), or on pantothenic acid.

No reports were given on the following general subjects: naval stores, radioactivity (including quantum counter, and analysis by radon measurement and alpha particle counting), and spectrographic methods.

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* For report of Subcommittee A and action by the Association see *This Journal*, **30**: 44 (1947). The methods are given in detail in *This Journal*, **30**: 84 (1947) under "Changes in Methods of Analysis."

REPORT ON PROCESSED VEGETABLE PRODUCTS

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

RECOMMENDATIONS*

It is recommended—

(1) That studies of methods for quality and fill of container be continued.

(2) That studies be continued on determination of moisture in dried vegetables.

(3) That the method for determination of catalase in frozen vegetables recommended by the Associate Referee be adopted as tentative.

(4) That studies for methods for determination of enzymes in frozen vegetables be continued.

No reports were given on quality factor, or moisture in dried vegetables.

PRELIMINARY REPORT ON DEVELOPMENT OF METHODS FOR CATALASE DETERMINATION IN FROZEN VEGETABLES

By HANS LINEWEAVER, *Associate Referee*, and HERMAN J. MORRIS (Western Regional Research Laboratory,† Albany, Calif.)

The method to be described was developed from existing methods primarily for the determination of catalase in frozen vegetables. However, the method, with only minor modification, may be used for the quantitative determination of catalase in many materials—for example, the determination of catalase in cereals, and for research purposes.

Lack of time has prevented comparison of this method with the large numbers of qualitative and semiquantitative methods for catalase that have been described in the literature. This method is fairly simple, is highly reliable, requires only ordinary laboratory equipment and no special training. It is not as simple as some methods (for example, those based on the development of O_2 gas) but is about ten times as sensitive as a method that uses the fermentation tube. It is about as sensitive as a method that uses the Warburg or similar apparatus; and it has the further advantage that confusion caused by air bubbles, as distinguished from liberated oxygen, is avoided. Tests on single lots of peas, string beans, cabbage, asparagus, and carrots showed that the proposed method will be

* For report of Subcommittee C, and action by the Association, see *This Journal*, 30: 49 (1947).

† Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

positive if more than 0.5 per cent of the catalase originally present in the vegetable remains in the frozen product. This method should be especially useful as an "umpire" method.

The method is basically that described by Jolles (1), and by Balls and Hale (2). In principle, the rate of hydrogen peroxide decomposition is determined from measurements of the residual hydrogen peroxide. The methods of Jolles, and of Balls and Hale call for thiosulfate titration of iodine liberated from potassium iodide by the residual hydrogen peroxide. The presence of considerable amounts of soluble starch (commonly present in extracts of many blanched vegetables) obscures the end point. This difficulty is avoided in the present method by addition of sodium thiosulfate to the acid mixture to react with the iodine as it is liberated; the excess sodium thiosulfate is determined by titration with iodine. The increase in iodine titer value is a direct measure of the amount of hydrogen peroxide decomposed in the corresponding time interval.

The details of the method are described in *This Journal*, **30**: 76 (1947) under "Changes in Methods of Analysis."

RESULTS OF COLLABORATIVE STUDIES

One sample each of well-blanched peas, string beans, and spinach were analyzed in four different laboratories. Duplicate extracts and duplicate

TABLE 1.—*Summary of catalase results on well-balanced vegetables*[1]

	PEAS	SPINACH	STRING BEANS
	ml of I	*ml of I*	*ml of I*
Titer values 0 to 5′			
range	−0.18 to 0.30	−0.20 to 0.10	−0.15 to 0.10
average	0.06	−0.01	−0.02
Standard deviation	0.13	0.09	0.07
Standard error of average	0.03	0.02	0.02
Titer values 0 to 10′			
range	−0.20 to 0.50	−0.05 to 0.15	−0.25 to 0.08
average	0.14	0.03	−0.05
Standard deviation	0.20	0.06	0.10
Standard error of average	0.05	0.025	0.025
Catalase test	possibly trace[2]	negative	negative

[1] The results of two of the collaborators who received their samples late were not included in this tabulation. However, their results are in agreement with the results of the other three collaborators and our own.

[2] A positive test for inspection purposes was recorded for only one extract (average of duplicate runs) and only for the 10-minute time interval; the 5-minute interval gave an average titer difference of only 0.10 ml, corresponding to a negative test.

runs on each extract were made. Table 1 shows that with the possible exception of peas catalase was absent in all cases. For processing control or inspection purposes a sample giving titer value differences of less than

0.2 and 0.4 ml. of iodine solution for the 5- and 10-minute time intervals, respectively, should be considered to have practically no catalase. This will correspond to more than 99.5 per cent inactivation of catalase in the case of all vegetables so far tested.

Table 2 shows the 0- to 5-minute titer differences for a sample of mildly blanched string beans in which about 97 per cent of the catalase activity had been destroyed. The differences are large even when only 3 ml. of extract was used; the value obtained in this case obviously corresponds to the value that would be obtained with 10 ml. of extract and about 99 per

TABLE 2.—*Catalase activity of mildly-blanched string bean sample*

ANALYST[1] NO.	AMOUNT OF EXTRACT	TITER DIFFERENCES 0 TO 5′	TEMPERATURE	H_2O_2 EQUIVALENT		K'_f[2]
				$t=0$	$t=5'$	
	ml	*ml of I*	°C.	*ml of I*	*ml of I*	
1	3	0.74	20	3.68	2.93	0.026
1	3	0.92	18	3.66	2.74	0.033
2	10	2.32	18	4.75	2.43	.023
2	10	2.37	18	5.00	2.62	.022
3	10	2.28	11	3.78	1.50	.032
3	10	2.05	16	3.73	1.67	.028
4	5	1.80	15	4.65	2.85	.034[3]
4	5	1.66	15	4.91	3.26	.028

[1] See footnote 1, Table 1.
[2] K'_f is the catalase activity (Katalase fahigkeit) per gram of sample at zero degrees; K'_f is defined for this report as the catalase activity at the experimental temperature.
[3] Single run. Other values are the average of duplicate runs on single extracts.

cent inactivation. It should be noted also that in spite of differences in amount of extract used, differences in temperature, and differences in the hydrogen peroxide equivalent at $t=0$, the K'_f values do not differ markedly. It appears that relative catalase activities may be estimated without working at zero degrees, which, of course, must be used to obtain K'_f, since it is defined as the activity at zero degrees.

SUMMARY AND CONCLUSIONS

A method has been described for determining catalase in frozen vegetables. It has been adapted from a well-known titrimetric method, in which the undecomposed hydrogen peroxide is determined after reaction for 5- and 10-minute intervals. It requires only ordinary equipment and skill.

It is more reliable and more sensitive than most gasometric methods for catalase. It may be used qualitatively by setting a tolerance, which would include experimental error, or quantitatively by only slight modification of technique. It therefore is of general usefulness.

Six collaborative laboratories reported catalase absent by this method in well-blanched string beans, spinach, and peas. They also reported high

catalase activities in mildly blanched vegetables, in which only 70 to 97% of the catalase was destroyed.

COLLABORATORS

The generous cooperation of the following collaborators is greatly appreciated:

Mrs. Irene Breschini and Mr. Eric Todd, who are connected with commercial firms.

J. Wm. Cook, Food and Drug Administration, Federal Security Agency.

K. C. Kimball, Processed Products Inspection, Production and Marketing Administration, U. S. Department of Agriculture.

M. P. Masure, Food Products Division, Western Regional Research Laboratory, U. S. Department of Agriculture.

Summary of Comments by Collaborators: All collaborators felt that the method was sound. It was pointed out, however, that a more rapid method would be desirable, not only for enzyme control at the processing lines but also for inspection work.

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No report was given for the general subject fill of container methods (food, drugs, and cosmetics).

REPORT ON COFFEE AND TEA

By H. J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Referee*

At the 1939 meeting the Referee recommended[1] that methods for the determination of chlorogenic acid in coffee be studied. Such a study required a supply of pure chlorogenic acid or one of its salts. The Referee has tried unsuccessfully since that date to obtain a sample of pure chlorogenic acid. In 1941 he received a communication from the H. S. Polin Laboratories of New York City in which they offered to supply him with chlorogenic acid and stated that they had devised a method of their own for its determination, but in spite of further correspondence neither the chlorogenic acid nor a copy of the method was ever received. Apparently before work on chlorogenic acid methods can be undertaken it will be necessary for the Referee to isolate his own chlorogenic acid from raw coffee. Details of this separation have been outlined by Plücker and Keilholz.[2]

[1] *This Journal*, **23**, 606 (1940).
[2] *Z. Unt. Lebensm.*, **66**, 200 (1933).

In the course of editing the Coffee and Tea chapter for the sixth edition of *Methods of Analysis* the Referee's attention was called to the fact that the Bailey-Andrew method (**18.41**) had been approved by the Association only as a method for the determination of caffeine in tea. No collaborative work had ever been done on its application to the determination of caffeine in coffee. Since the method had been used successfuly at the Connecticut Agricultural Experiment Station for determining caffeine in coffee, and since it is simpler and more expeditious than the present official and tentative methods for coffee, it appeared desirable that a collaborative study of the Bailey-Andrew method as applied to coffee be undertaken. On the Referee's recommendation Mr. Gilman K. Crowell was appointed to study this problem. He submitted to five collaborators two samples of coffee extracts for determination of caffeine by the Bailey-Andrew (**18.41**) and Fendler-Stüber (**18.15**) methods and by a modification of the Power-Chesnut method (**18.14**). No significant differences were found between results by the three methods. His report has been published.[3]

All collaborative work was done on coffee extracts and none on straight coffee. Since earlier work of Crowell[4] had shown, however, that the Fendler-Stüber method, which has already been adopted as a tentative method for straight coffee, gave satisfactory results on coffee extracts containing added carbohydrates, while the unmodified Power-Chesnut method did not, and that the Bailey-Andrew method was equally satisfactory, the evidence seems sufficient for recommending the Bailey-Andrew method as a method for straight coffee. Mr. Crowell has also devised a modification of the Power-Chesnut method that yields satisfactory results with coffee extracts, but since both the Fendler-Stüber and Bailey-Andrew methods, which are simpler, can be applied to coffee extracts without modification, it does not seem worth while to recommend adoption of the modified Power-Chesnut method.

RECOMMENDATIONS*

It is recommended—

(1) That the phrase "Not applicable to coffee extracts" be inserted below the title of the Power-Chesnut method (**18.14**). (First action.)

(2) That the Fendler-Stüber method (modified) (**18.15**) be made official (first action).

(3) That the Bailey-Andrew method for caffeine in tea (**18.41**) be adopted as an official method (first action) for caffeine in coffee.

(4) That the study of methods for the determination of chlorogenic acid in coffee be reassigned.

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[3] *This Journal*, **29**, 37 (1946).
[4] *Ibid.*, **27**, 168 (1944).
* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 49 (1947).

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (Food and Drug Administration, New York, N.Y.), *Referee*

The Referee has not been able to complete any of the problems recommended by the Association for the current year. Causes over which no control could be exercised nor previously foreseen hampered progress of the investigations.

However, considerable advance can be reported for the differentiation between Orange SS, FD&C Orange No. 2, and Oil Red XO, FD&C Red No. 32.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the detection of small (or large) amounts of Tartrazine FD&C Yellow No. 5 in alimentary paste be submitted to further collaboration.

(2) That investigational work be continued on the quantitative separation and estimation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow F.C.F.)

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. Yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow A.B.), FD&C Yellow No. 4 (Yellow O.B.), FD&C Orange No. 2 (Orange S.S.), and FD&C Red No. 32 (Oil Red X.O.).

(5) That collaborative work on analytical methods for coal-tar colors certifiable for use in foods be conducted. This general subject parallels to some degree studies in Coal-tar Colors being pursued under Committee B. Committee C suggests that the Chairman of the Committee on Recommendations of Referees assign the subject to prevent duplication.

REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemical Laboratory, Vermillion, South Dakota), *Referee*

It has not been possible to accomplish since our last previous meeting all of the studies that have come to mind or have been suggested by others relative to the methods for dairy products. However, some very creditable work has been done in spite of shortage of help and of pressure of other

* For report of Subcommittee C and action by the Association, see *This Journal*, 30: 49 (1947).

duties. Notable are the studies in three different laboratories on the tests for that elusive enzyme, phosphatase, which occurs in milk. Sanders and Sager[1] developed a method applicable to cheddar cheese and have since modified their procedure so as to make it of broader value. Gilcreas, Associate Referee on phosphatase test for hard cheese, has conducted collaborative studies in which a modified Kay-Graham procedure and the Sanders-Sager method were compared by a large number of workers. This study demonstrated that the Kay-Graham procedure could not be "satisfactorily adapted to the examination of cheese." The Sanders-Sager technic, on the other hand, was found reliable when applied to cheese and the Associate Referee recommended that it be adopted as a tentative procedure. Report of this work was presented at the 1946 meeting of the Association. Another worker, Horwitz, was appointed as Associate Referee on phosphatase test for soft cheese and he has published an article[2] describing modifications of a present tentative method for phosphatase test on milk[3] and also showing that with modifications the method may be successfully applied to soft cheeses.

It is believed that, as a result of these several studies of phosphatase test methods, it should be possible to work out a method applicable to all or nearly all dairy products. Therefore, it is the recommendation of your Referee that there be appointed an Associate Referee on the phosphatase test as an index of pasteurization, and that he study the development of a unified method applicable to milk and other dairy products.

The Associate Referee on sampling, fat, and moisture in cheese has called attention in his report to a simplified procedure for fat in cheese. The changes suggested for this method appear to offer advantages over the present method. I recommend, therefore, that an Associate Referee be appointed to study possible improvements in the method for fat in cheese.

A paper from the Referee's laboratory,[4] read at this meeting, details the results of a study of the method for determining acidity of milk. It is shown that close attention to degree of dilution of sample and of indicator are required to obtain the correct *p*H value to insure accurate results. It is recommended, therefore, that collaborative work be conducted to study the determination of the acidity of milk.

Although the Associate Referee on ice cream was not able to submit a report of progress, he did make two recommendations. Your Referee approved the second of these and recommends that studies be undertaken on quantitative determination of gelatin and other stabilizers in frozen desserts.

The Referee recommends that study of methods for detection of re-

[1] *This Journal*, **28**, 656 (1945).
[2] *Ibid.*, **29**, 129 (1946).
[3] *Ibid.*, **24**, 76 (1941).
[4] "Determination of the Titrable Acidity of Milk," by E. H. Zilliox, D. J. Mitchell, and Guy G. Frary, *This Journal*, **30**, 130 (1947).

constituted milk and of methods for detection of chlorine in milk be continued. The work of Wilson[5] on quaternary compounds in foods has developed a method said to be applicable to detection of such substances in milk. Recent communication from a State health official indicates that such preparations may be finding their way into commercial milk supplies, as evidenced by the occurrence of increasing numbers of zero plate counts on market milk. It is recommended, therefore, that an Associate Referee be appointed to study methods for the detection and estimation of quaternary compounds in milk.

The Associate Referee on sampling of butter was not able to make a report for this meeting but he has been making active study of this vexing but none-the-less very important problem, as reported in *This Journal*, **29**, 119 (1946). I recommend that the study be continued.

RECOMMENDATIONS*

It is recommended—

(1) That there be appointed an associate referee on the phosphatase test as an index of pasteurization, and that he study the development of a unified method applicable to milk and other dairy products.

(2) That methods for the detection of reconstituted milk be studied.

(3) That studies be continued on a quantitative method for gelatine and other stabilizers in frozen desserts.

(4) That studies be continued on chlorine in milk.

(5) That studies on methods for fat, moisture, and sampling of cheese be continued.

(6) That study of methods for acidity of milk be continued.

(7) That studies be continued on methods for the preparation of samples of butter.

(8) That the tentative method for ash in milk (**22.16**) be further studied with a veiw to adoption as official.

(9) That the official, first action, method (**22.146b**) for the preparation of sample of frozen desserts be further studied.

(10) That an associate referee be appointed to study methods for ice cream and frozen desserts.

(11) That the method for sour serum test be investigated.

No report was made on pasteurization of milk and cream or on ash in milk and evaporated milk.

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[5] *Ibid.*, 29, 311 (1946).

* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 50 (1947).

REPORT ON SAMPLING, FAT, AND MOISTURE IN CHEESE

By William Horwitz (U. S. Food and Drug Administration, Federal Security Agency, Minneapolis 1, Minnesota),
Associate Referee

The distribution of moisture and fat in a cheese is of fundamental importance to the sampling procedure. It is, therefore, necessary to first determine what this distribution is before consideration can be given as to how the cheese should be sampled. With this in mind, eight small cheeses of the cheddar variety were prepared in a cheese plant and were placed in storage under commercial conditions. At various intervals, a cheese was withdrawn, cut up into 400 uniform sections, and each section ground and analyzed for moisture. Up to the present time three cheeses have been analyzed, but since the study is not complete and since the data have not been analyzed statistically, a report is not presented. The individual moisture results will be subjected to statistical analysis in an attempt to predict just where and how a core should be taken which will give a moisture figure approximating the average moisture content of the entire cheese. After the analysis has predicted where the sampling core should be taken, a large number of cheeses will be sampled at this point and the results for fat and moisture will be compared with the results on a representative sample of the corresponding ground whole cheese.

Before this project could be undertaken, however, a much more rapid method for the moisture determination in cheese than the present official vacuum oven method had to be found. Such a method (unpublished) was provided by E. F. Steagall of the Food Division, U. S. Food and Drug Administration, who showed that cheese dried in the forced draft oven at 130°C. for one to one and one-half hours gave results comparable to vacuum oven drying at 100°C for four hours. This method was not intended to replace the present official method, but was designed primarily for rapid results where only relative data were desired.

Previous unpublished experiments by the Associate Referee had shown that the temperature distribution in the forced draft oven was rather uniform with variations of the order of magnitude of plus or minus 1°C on a single shelf or from shelf to shelf.

Included in the unpublished reports by the previous Associate Referee, J. B. Snider, was a simplified version of the present official method for fat in cheese. This method eliminates the transfer of the digested cheese from a beaker to the extraction flask. It is based upon a procedure, devised by Ferris of the Buffalo Station of the U. S. Food and Drug Administration, using a special vessel which combined in a single unit the flat bottom of a beaker for the digestion with the trap and the pouring features of the Mojonnier tube for the extraction. The method as proposed by Snider is as follows:

Weigh ca 1 g cheese directly into a tared Mojonnier tube using a long handled trough spatula, taking care that no particles are left on the neck. Add 10 ml NH_4OH (1+9), washing down the sides of the tube. Place in a hot water bath and shake frequently until the casein is well softened. Cool slightly, add 11 ml HCl, mix, and heat in the boiling water bath until the casein is digested and the liquid has darkened (15–40 min). Shake occasionally to wash down particles in the upper part of the tube. When digestion is complete, cool and extract fat according to *Methods of Analysis*, 1945, sec. **22. 130.**

Snider reported the results of fat on the dry basis of 29 different samples by the proposed method and by the official method. The average fat content of all the samples by one method was identical with that obtained by the other. This method has been used repeatedly in this laboratory as a check against the official method with excellent results. The method appears promising and collaborative work should be performed with it.

It is recommended* that studies on sampling, fat, and moisture in cheese be continued.

REPORT ON FROZEN DESSERTS

By F. Leslie Hart (Food and Drug Association, Federal Security Agency, Los Angeles 15, Calif.), *Associate Referee*

RECOMMENDATIONS†

It is recommended—

(1) That the method for preparation of sample of frozen desserts containing insoluble ingredients, *Methods of Analysis* **22. 146b,** be adopted as official.

(2) That studies be undertaken on quantitative determination of gelatin and other stabilizers in frozen desserts.

No reports were given on chlorine in milk, acidity of milk, or preparation of butter samples.

REPORT ON TESTS FOR PASTEURIZATION OF DAIRY PRODUCTS—THE PHOSPHATASE TEST IN THE EXAMINATION OF HARD CHEESE

By F. W. Gilcreas (Division of Laboratories and Research, New York State Department of Health, Albany 1, N. Y.), *Associate Referee*

Methods for determining the inactivation of the enzyme phosphatase in milk indicate the degree of heat-treatment precisely and have become

* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 49 (1947).
† For report of Subcommittee C and action of the Association, see *This Journal*, **30**, 50 (1947).

indispensable in the control of pasteurization. Adaptation of these procedures to the examination of other dairy products has frequently been necessary. New York and many other States now require that cheese be made only from pasteurized milk or be aged for sixty days (1). This regulatory action has increased the need for methods that will indicate the heat-treatment given the milk from which the cheese has been prepared. The original Kay-Graham test (2) and modifications developed in the New York City Department of Health (3) have been used for this purpose. Recently, Sanders and Sager of the Bureau of Dairy Industry of the United States Department of Agriculture have presented a method for determination of the degree of heat-treatment of hard cheese, 2,6-dibromoquinonechloroimine (BQC) being used as indicator (4). A comparative study with the phosphatase test was therefore important so that a standard procedure might be established. It was necessary not only to investigate the precision of the methods in detecting the presence of an active enzyme but also to determine the effect of possible interference by products of proteolytic decomposition, particularly in cheese that has been aged for considerable periods.

The principal obstacle in the way of the cooperative study was that of obtaining samples of cheese with a known history of manufacture and known periods of aging. Through the kindness of Dr. A. C. Dahlberg of the College of Agriculture, Cornell University, a series of such samples was made available. They represented cheese prepared from raw milk, underpasteurized milk, and pasteurized milk; all were aged for periods ranging from three months to four years. Each set of three types of cheese had been prepared from milk from the same source. In addition, several samples of cheese were prepared by mixing a portion made from pasteurized milk and that made from raw milk to provide proportions of 1 per cent, 0.6 per cent, and 0.3 per cent of raw-milk cheese combined with the pasteurized product. Each sample was run through a small meat grinder several times, and the degree of mixing was checked by selecting and testing samples of the mixed portion. Thus the uniformity of small amounts submitted as samples to the collaborating laboratories was assured. Thirteen laboratories examined the series of 19 samples identified only by serial number:

Bureau of Laboratories, Connecticut Department of Health, Hartford, Conn., Friend Lee Mickle, Director.

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C., George P. Sanders, Chemist.

Food and Drug Administration, Federal Security Agency, Chicago Station, Chicago, Ill., Iman Schurman, Acting Chief.

Food and Drug Administration, Federal Security Agency, New Orleans Station, New Orleans, La., George McClellan, Analyst.

Division of Chemistry, Bureau of Laboratories, Baltimore City Health Department, Baltimore, Md., Emanuel Kaplan, Chief.

Food and Drug Administration, Federal Security Agency, Boston Station, Boston, Mass., Andrew M. Allison, Acting Chief.

Food and Drug Administration, Federal Security Agency, Minneapolis Station, Minneapolis, Minn., William Horwitz, Chemist.

Food and Drug Administration, Federal Security Agency, St. Louis Station, St. Louis, Mo., Samuel Alfend, Acting Chief.

Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. (Analyst 1).

Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. (Analyst 2).

Food and Drug Administration, Federal Security Agency, Buffalo Station, Buffalo, N. Y., Leslie W. Ferris, Chemist.

Food and Drug Administration, Federal Security Agency, New York Station, New York, N. Y., Gardner Kirsten, Chemist.

Food and Drug Administration, Federal Security Agency, Cincinnati Station, Cincinnati, Ohio, Sam D. Fine, Chemist.

Department of Dairy Technology, Ohio State University, Columbus, Ohio, L. H. Burgwald.

State Chemical Laboratory, Vermillion, S. Dak., Guy G. Frary, State Chemist.

Two technics were selected: A. A slight modification of the original Kay-Graham method, by which cheese following maceration and preparation could be examined. B. The Sanders-Sager technic as submitted by the authors (5). The samples were forwarded to each of the collaborating laboratories on August 9, 1946; three weeks were allowed to complete the examination by both technics, including determination of control values for each test. The use of either visual comparison with permanent standards or determination of color in a photoelectric colorimeter was permitted.

The results reported by the 15 collaborators—13 laboratories plus determinations made in the laboratory of the Referee by two different analysts, are given in Tables 1 and 2. In addition, the results by the Sanders-Sager procedure are shown graphically in order to permit appraisal of the variations in color readings.

DISCUSSION

The collaborative study indicates that with method A, the modified Kay-Graham determination, the control values, particularly in the examination of aged cheese, are so high as to limit sharply the utility of this test for detecting the presence of the active enzyme in the sample. This interference is undoubtedly caused by amino acids, particularly tyrosine, which are always present in aged cheese. Routine application of the modified Kay-Graham procedure in the Referee's laboratory to freshly made cheese indicated that interference by proteolytic products is limited and that the test can be used with precision on such samples. Tables 1 and 2 show that in several laboratories the control values obtained with this technic were so high that study of the test on the remaining samples was abandoned. The Sanders-Sager method, however, in 135 examinations

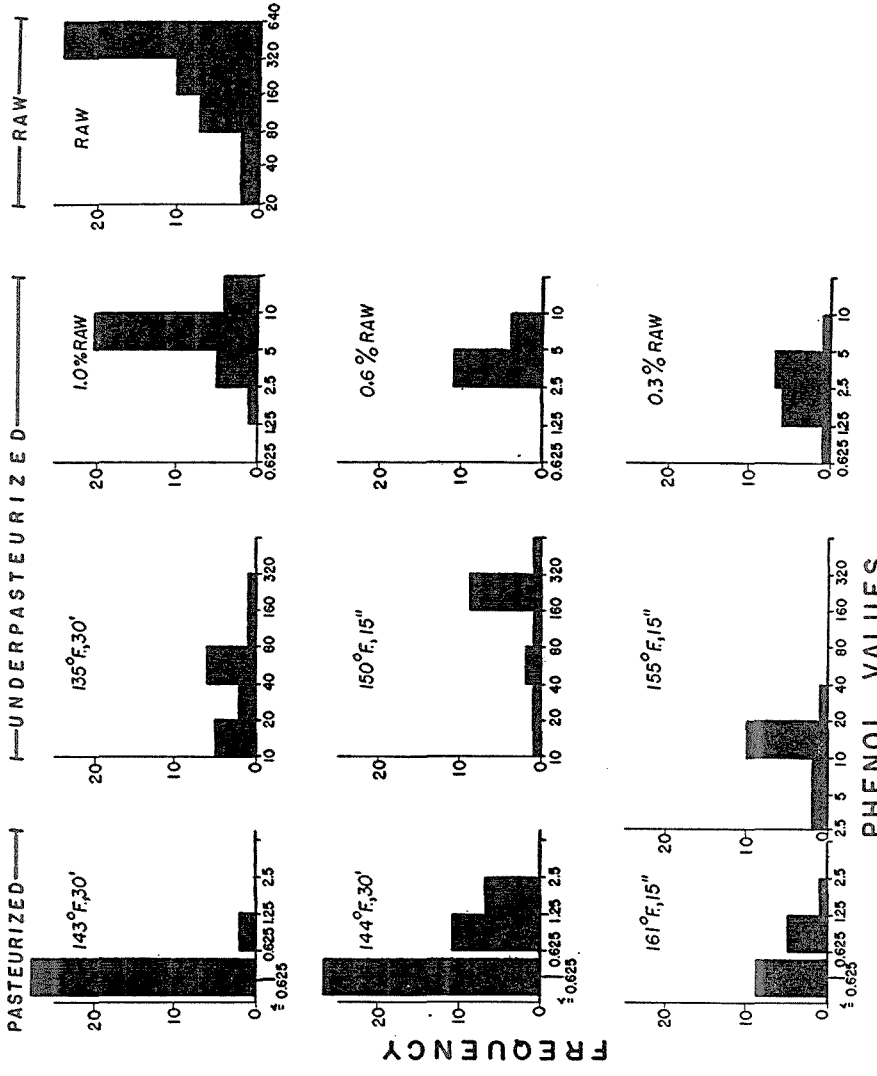

FIG. 1.—Frequency distribution of observed phenol values in the examination of hard cheese by the Sanders-Sager method (expressed as micromilligrams of phenol per 0.25 gram of sample).

TABLE 1.—*Examination of hard cheese by the Sanders-Sager method—Color unit readings of samples reported by collaborating laboratories*
(Readings expressed as micromilligrams of phenol per 0.25 gram of sample)

SAMPLE NO.	1		2		3		4		5		6		7		8		9		10	
HEAT-TREATMENT DATE MANUFACTURED	150°F.—15′ 1-22-43		155°F.—15′ 1-22-43		161°F.—15′ 1-22-43		0.3% RAW (PASTEURIZED PROCESS+RAW) MIXED 8-1-46		RAW 2-28-44		143°F.—30′ 2-28-44		1% RAW MIXED 8-1-46		135°F.—30′ 2-28-44		0.6% RAW MIXED 8-1-46		RAW 7-16-45	
Identification No. of laboratory	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*
1	200	0	18		1.5		5		180	0	0		10		20		10		400	0
2	348	0	18.4	0	1.2	0	3.3	0	423	0	0.7	0	8.0	0	173	0	6.1	0	585	0
3	200	0	10	0	0.5	0.5	7	0	400	0	0	0.5	11	2	19.5	0.5	4	2	149.5	0.5
4	240	0	15	0	1	0	2	1	150	0	0	0	8	1	80	0	4	1	400	1
5	250	0	15	0	0	0	2	0	250	0	1	0	8	0	60	0	6	0	300	0
6	27.1	1.1	11.5	0.7	1.0	0.6	2.5	1.2	20.1	1.3	0.4	1.2	5.2	2.1	18.6	1.3	3.1	1.6	31.1	0.7
7	240	0	13	0	0.5	0	3.5	0	360	0	0	0	11	0	25	0	4.5	0	360	0
8	250	0	20	0	1	0	3	0	250	0	0	0	8	0	50	0	5	0	375	0
9	20	0	10		0		2		60	0	0		5		20		4		100	0
10	240	0	14*	0	0.5	0	4	0	400	0	0	0	10	0	26*	0	6*	0	400	0
11	75	0	3	1	0	1	2	1	100	0	0	0	4	1	12	1	4	1	100	0
12	240	0	12	0	0	0	1	0	320	0	0	0	7	0	160	0	3.5	0	384	0
13	256	0	24	0	0	0	2	0	224	0	0	0	6	0	68	0	3	0	400	0
14	125	0	5	0	0	0	3	0	250	0	0	0	5	0	50	0	4	0	375	0
15	60	0	20	0	1	0	4	0	120	0	0	0	10	0	60	0	5	0	640	0

* Average of two values submitted.

TABLE 1.—(*continued*)

SAMPLE NO.	11		12		13		14		15		16		17		18		19	
HEAT-TREATMENT DATE MANUFACTURED	144°F.—30′ 7-9-45		144°F.—30′ 7-16-45		RAW 7-9-45		144°F.—30′ 7-9-45		144°F.—30′ 7-9-45		144°F.—30′ 5-10-46		1% RAW DUPLICATE #7		143°F.—30′ DUPLICATE #6		144°F.—30′ DUPLICATE #12	
Identification No. of laboratory	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*
1	1.0	0.5	0.5	0.5	400	0	1.5		1.0		1.0		10		0		0.3	0.5
2	1.3	0	1.5	0	600	0	0.3	0	0.6	0	0	0	7.7	0	0.1	0	0.7	0
3	1.5	0	0	0.5	450	0	1	0	0.5	0	1	1	1.5	2.5	0	0	0	0
4	0	0	1	0	490	0	0	0	0	0	0	1	7	1	0	0	0	0
5	2	0	1	0	300	0	2	0	0.5	0	1	0	8	0	0	0	0.5	0
6	0	0.7	0.4	1.1	42.4	1.2	0.8	0.7	0.5	2.2	0.5	1.9	9.0	0.9	0.5	0.9	0.6	1.4
7	0.5	0	0.2	0	360	0	0.5	0	0.5	0	0.7	0	11	0	0	0	0.2	0
8	1	0	1	0	300	0	1	0	0	0	1	0	7	0	0	0	1	0
9	0		0		120	0	0		0		0		7.5		0		0	0
10	1	0	0.5	0	500	0	0.5	0	1	0	0.5	0	11*	0	0	0	0.5	0
11	0	1	0	1	450	0	0	1	1	1	0	1	7	1	0	0	0	1
12	0	0	0	0	320	0	0	0	0	0	0	0	6	0	0	0	0	0
13	0	0	0	0	352	0	0	0	0	0	0	0	5	0	0	0	0	0
14	0	0	0	0	375	0	0	0	0	0	1	0	5	0	0	0	0	0
15	2	0	1	0	480	0	1	0	1	0	1	0	10	0	0	0	0	0

TABLE 2.—*Examination of hard cheese by the modified Kay-Graham method—Color unit readings of samples reported by collaborating laboratories*

(Readings expressed as milligrams of phenol)

SAMPLE NO.	1		2		3		4		5		6		7		8		9		10	
HEAT-TREATMENT DATE MANUFACTURED	150°F.—15″ 1-22-43		155°F.—15″ 1-22-43		161°F.—15″ 1-22-43		0.3% RAW (PASTEURIZED PROCESS+RAW) MIXED 8-1-46		RAW 2-28-44		143°F.—30′ 2-28-44		1% RAW MIXED 8-1-46		135°F.—30′ 2-28-44		0.6% RAW MIXED 8-1-46		RAW 7-16-45	
Identification No. of laboratory	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*
1		>0.15		>0.15		>0.15		0.07		>0.15		>0.15		0.07		>0.15		0.07		>0.15
2	0.514	0.262	0.356	0.268	0.048	0.196	0.034	0.086	0.584	0.228	0.046	0.148	0.08	0.068	0.384	0.249	0.04	0.066	0.548	0.233
3	0.54	0.18	0.34	0.28	0.12	0.16	0.08	0.05	0.62	0.20	0.04	0.14	0.03	0.08	0.17	0.30	0.05	0.07	0.57	0.25
4	0.57	0.35	0.22	0.38	0	0.24	0.04	0.06	0.60	0.32	0.02	0.23	0.05	0.06	0.49	0.31	0.02	0.08	0.60	0.20
6	0.33	0.13	0.19	0.16	0.02	0.19	0.02	0.08	0.28	0.16	0.02	0.13	0.05	0.06	0.16	0.15	0.02	0.06	0.15	0.14
10						0.083		>0.15					0.048	0.081			0.01	0.087		
12	>0.44	0.23	>0.26	0.33	0.04	0.23	0.06	0.06	>0.34	0.33	0.03	0.13	0.10	0.05	>0.34	0.33	0.07	0.05	>0.47	0.20
13	>0.41	0.26	>0.45	0.22	0.11	0.26	0.06	0.05	>0.33	0.34	0.02	0.20	0.11	0.06	>0.30	0.37	0.08	0.06	>0.41	0.26

SAMPLE NO.	11		12		13		14		15		16		17		18		19	
HEAT-TREATMENT DATE MANUFACTURED	144°F.—30′ 7-9-45		144°F.—30′ 7-16-45		RAW 7-9-45		144°F.—30′ 7-9-45		144°F.—30′ 7-9-45		144°F.—30′ 5-10-46		1% RAW DUPLICATE #7		143°F.—30′ DUPLICATE #6		144°F.—30′ DUPLICATE #12	
Identification No. of laboratory	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*	*Sample*	*Control*
1		>0.15		>0.15		>0.15		>0.15		>0.15		0.05		0.07		>0.15		>0.15
2	0.032	0.171	0	0.190	0.582	0.246	0.011	0.177	0.020	0.199	0.015	0.033	0.062	0.069	0.017	0.183	0.017	0.177
3	0.02	0.14	0.04	0.13	0.74	0.18	0.02	0.20	0.04	0.14	0.02	0.025	0.11	0.06	0.12	0.15	0.02	0.18
4	0.03	0.17	0.07	0.17	0.82	0.30	0.03	0.19	0.08	0.17	0.01	0.03	0.09	0.04	0.01	0.17	0.03	0.20
6	0.02	0.12	0.02	0.12	0.19	0.13	0.05	0.11	0.04	0.11	0.04	0.03	0.07	0.04	0.04	0.10	0.03	0.10
10											0.015	0.055	0.037	0.080				
12	0.07	0.15	0.02	0.20	>0.34	0.33	0.03	0.22	0.03	0.22	0.03	0.025	0.10	0.05	0.02	0.20	0.02	0.21
13	0.02	0.25	0.02	0.25	>0.34	0.33	0.02	0.25	0.02	0.21	0.02	0.03	0.08	0.09	0.03	0.20	0.03	0.22

of cheese prepared from pasteurized milk, consistently yielded results of less than 2.5 units for perfectly pasteurized cheese. The number of color units in samples of grossly underpasteurized or raw cheese varied widely. This is undoubtedly due to the method of diluting the color obtained on

TABLE 3.—*Sanders-Sager method—Reading of dilutions of phenol values greater than 30 color units*

SAMPLE NO.	DILUTION	DIRECT READING	READING CORRECTED BY FACTOR OF DILUTION
		*Color units**	*Color units**
	1–5	15	75
	1–8	17	136
1	1–16	15	240
	1–25	4	100
	1–32	3	96
	1–5	14	70
	1–8	17	136
5	1–16	20	320
	1–25	4	100
	1–32	10	320
	1–5	14	70
	1–8	15	120
8	1–16	10	160
	1–25	4	100
	1–32	3	96
	1–5	25	125
	1–8	25	200
10	1–16	30	480
	1–25	10	250
	1–32	12	384
	1–5	20	100
	1–8	22	176
13	1–16	20	320
	1–25	10	250
	1–32	7	224

* Unit = micromilligrams of phenol per 0.25 gram of cheese.

examination of such samples in order to secure a color within the range of the standards.

In considering these discrepancies a brief experiment was undertaken in the Referee's laboratory to determine the effect of the method of dilution on the color (see Table 3). This showed that the method of dilution had a marked effect upon the magnitude of the reading in phosphatase units on

samples of cheese made from grossly underpasteurized or raw milk. However, this is of no practical importance since the precise reading of the number of units represented by colors greater than that of the No. 30 standard is of little significance because this color, or units greater than it, would indicate gross underpasteurization. The control values on the Sanders-Sager technic varied slightly but indicate no significant interference with the method by products of proteolytic digestion.

SUMMARY AND RECOMMENDATION

The Kay-Graham test could not be satisfactorily adapted to the examination of cheese.

The Sanders-Sager technic (4, 5) provides a reliable estimate of the inactivation of the enzyme phosphatase and thus of the degree of heat treatment of the milk from which hard cheese has been made.

It is, therefore, recommended* that the Sanders-Sager method, using either permanent standards or a photoelectric colorimeter to determine the phenol value, be adopted by the Association of Official Agricultural Chemists as a tentative standard procedure for determination of the degree of pasteurization of hard cheese.

REFERENCES

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(2) KAY, H. D., and GRAHAM, W. R., Jr., The phosphatase test for pasteurized milk. *J. Dairy Research*, **6**, 191–203 (1935).
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(4) SANDERS, G. P., and SAGER, O. S., Development of a phosphatase test applicable to cheddar cheese. *This Journal*. **28**, 656–675 (1945).
(5) *J. Dairy Sci.*, **29**, 737 (1946).

REPORT ON PASTEURIZATION TEST FOR SOFT CHEESES

BY WILLIAM HORWITZ (U. S. Food and Drug Administration, Federal Security Agency, Minneapolis 1, Minn.),
Associate Referee

The Associate Referee has published a modified Scharer phosphatase test directly applicable to the soft cheeses[1]. While this material was in press Sanders and Sager of the Bureau of Dairy Industry developed an improved version of their original modification of the Scharer procedure,[2] designed for the hard cheeses. The improvements made by Sanders and Sager resulted in a lower blank reading and an increase in the

* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 50 (1947).
[1] *This Journal*, **29**, 129 (1946).
[2] *This Journal*, **28**, 656 (1945). Since this paper was presented Sanders and Sager published a further modification of their method in J. Dairy Sci., **29**, 737 (1946).

accuracy and precision. A few experiments made by the Associate Referee indicated that the new Sanders and Sager method could be applied to the soft cheeses with but minor modifications in the substrate buffer and protein precipitant concentrations. This procedure also had the advantage that it could become the basis of a "universal" phosphatase method applicable to all dairy products, since the incorporation of barium in the buffer substrate removes the phosphatase inhibitors (phosphates, citrates) which are normal constituents of some processed cheese products. In view of the desirability of a uniform phosphatase procedure, the Associate Referee discarded his method in favor of the Sanders and Sager procedure.

This change in phosphatase method, however, does in no way modify the conclusions reached in the earlier paper[1]. There is a definite relationship between the amount of phenol produced, when expressed in the same terms, by the method submitted by the Associate Referee and by the method of Sanders and Sager. The latter procedure results in an amount of phenol about 10 per cent greater than the former for a given milk or soft cheese sample. The important conclusion that cottage cheese, if prepared from properly pasteurized milk, should show a complete absence of phosphatase activity still holds true with the Sanders and Sager method.

The method submitted to the collaborators differed in a few respects from that used by Sanders and Sager. The changes were made after experience with the method indicated that they were more convenient or resulted in a simplification of the technique. In order to reduce the amount of work required, they were instructed to use a blank from U1 for all of the samples of the U series and a blank from C1 for all of the samples of the C series. The major differences from the method of Sanders and Sager[2] were in the use of an 8:2 dilution of the borate buffer for the substrate and a zinc-copper protein precipitant containing 4.5 g $ZnSO_4 \cdot 7H_2O$ and 0.15 g $CuSO_4 \cdot 5H_2O$ in 100 ml of water.

COLLABORATIVE SAMPLES

Seven samples of cottage and creamed cottage cheese were submitted to the collaborators. The C series consisted of three commercial samples of a homogeneous cottage cheese examined at this station during April and May, 1946, and placed in cold storage until mixed and sent out. The U series was prepared through the cooperation of the Dairy Department of the University of Minnesota. The milk for samples U1 and U3 contained 2 and 8 per cent raw skim milk, respectively, but unfortunately the curd had to be cooked to 130°F in 1½ hours before it became firm, so that these samples were practically devoid of phosphatase activity. Sample U2 was prepared from 5 pounds of the dry cottage cheese U1 by adding 1 ounce of salt and 2 pounds of pasteurized cream (15% fat) containing 1 ml of raw cream (40% fat). Sample U4 was prepared from 5 pounds of the dry cottage cheese U3 by adding 1 ounce of salt and 2

TABLE 1.—*Collaborative results on phosphatase in soft cheeses, in mmg/g*

COLLABORATOR	DATE		U1		U2		U3		U4		C1	C2	C3	
	RECD	ANAL	Bu	Aq	Bu	Aq	Bu	Aq	Bu	Aq	Aq	Aq	Bu	Aq
Assoc. Ref.		8/2	0.4		4.8		0.5		13.1		101	39.9	7.8	
		8/5	0.3		5.7		0.6		12.1		82	29.7	5.1	
		8/30	0.2		3.5		0.4		12.7		98	29 2	4.8	
1	7/31	8/1	0.8		3.2		0.6			17.5	103	36.8	6.4	
			0.8		3 3		0.5			17.3	91*	36.3	6.9	
2	7/31	8/2	0.5		4.1		1.4		12		96*	43	7.4	
			0.5		5.1		1.4		12		106*	46	7.4	
3	8/2	8/2	0.7	2.1	4.4	3.4	0.0	0.4	13.6	13.1		Moldy		
4	8/1	8/9	0.0	2	5	6	0.0	1	14	17	101	35		5
				3	5	7		2	13	17	98	33		5
5-a		8/21		3.2		7.4		3.7		18.4	88*	25.8		6.9
b				2.8		6.9		3.2		20.2	87*	27.6		6.0
6	8/2	8/16	1.4				1.4							
			0.7				0.0							
		8/28			4.6					16.1		Decomposed		
					5.1					16.1				
Average			0.6	2.6	4.5	6.1	0.6	2.1	12.8	17.0	96	34.8	6.5	5.7
Ave. Dev. mmg/g			0.3	0.5	0.65	1.2	0.4	1.1	0.6	1.2	6	5.2	1.0	0.7
Ave. Dev. %					14	20			5	7	6	15	15	15

* Diluted once.

pounds of pasteurized cream (15% fat) containing 3 ml of raw cream (40% fat). Sample U2 therefore contained approximately 0.03% raw 40% cream; U4 contained approximately 0.1% raw 40% cream.

The samples of the C series were removed from cold storage on July 26 and were allowed to thaw out in the refrigerator over the week end. The individual retail cartons were emptied into a large beaker and the cheese was mixed thoroughly with a spatula and with the mechanical butter stirrer before transferring to the individual shipping cartons.

Samples U1 and U3 were prepared on July 25 and placed in a cooler until July 29, when they were packed in shipping cartons without further mixing. Samples U2 and U4 were prepared on July 29 and were thoroughly mixed with a spatula and with the mechanical butter stirrer before transferring to the shipping cartons.

All of the samples in the shipping cartons were placed in a freezer overnight and were shipped out the next day under dry ice. Only enough dry ice could be obtained to keep the samples frozen for three days. Because of transportation delays some of the samples were not delivered until 7 days after shipment. Two collaborators reported spoilage and surface mold and did not run any of the samples. Two other collaborators found the C series decomposed or moldy but ran the samples. These results are reported separately.

No samples of cream cheese were submitted to the collaborators since commercial phosphatase positive products could not be located in over a year of intermittent sampling. Facilities were not available at the time the cottage cheese samples were prepared for the preparation of cream cheese. No difficulties have been encountered by the Associate Referee with this product and added phosphatase from raw milk has been recovered quantitatively.

COLLABORATIVE RESULTS

The data reported by the collaborators are listed in Table 1. In this table *Bu* designates a color reading in butyl alcohol while *Aq* designates a color reading in aqueous solution. Three results which are actually averages of duplicates or triplicates are reported by the Associate Referee, and they were run on different days to detect any changes in phosphatase activity on storage over the period covered by the analyses of the collaborators. There may have been a loss of phosphatase in samples C2 and C3, but this is ignored in the calculation of averages.

The apparently negative phosphatase samples U1 and U3 show a significantly higher phenol content when the colors are read in aqueous solution rather than in butyl alcohol, although in most cases the results are within the limit of 3 mmg/g set in the previous paper.[1] The results in butyl alcohol are about 2 mmg/g less than those in aqueous solution. It is, therefore, necessary to make an extraction mandatory for small

quantities of phenol rather than optional as the method was written. It has been the experience of the Associate Referee, however, that if the quantity of phenol present in the aliquot used for the color reading exceeds 5 mmg (about 25 mmg/g) the aqueous reading gives the same result as does the extraction reading.

In the case of three samples (U2, U4, C3) some data is reported by both methods. In the first two cases the average aqueous readings are higher than the average alcohol readings, while in the last case it is about the same. However, it is very difficult to draw any conclusions from these data since in most cases the collaborators ran the samples by only one method. Since each set of collaborative samples has been subjected to a unique history of storage conditions after shipment, changes may have occurred which would increase or decrease the phosphatase content.

Despite the perishable nature of the samples and the thawing and refreezing that some were subjected to, the over-all average deviation of 12 per cent is somewhat better than expected. It was believed that a 10 per cent deviation could be expected in the hydrolysis, a 5 per cent deviation in the color development and variability of the blanks, and a 5 per cent deviation from the inhomogeneity of the small sample (0.5 g) used in the test, when relatively large quantities of phenol were involved (50 mmg/g or more). For smaller quantities, the variations in the color readings of the blanks become the predominant source of error, the magnitude of which is 2–3 mmg/g regardless of the quantity of phenol present.

On the basis of the previous collaborative work on the Scharer phosphatase method for milk[3] where an over-all average deviation of about 25 per cent was considered a sufficient degree of reproducibility for adoption as a tentative method, the present method may also be considered satisfactory. Qualitatively, all samples including the borderline samples U2 and C3 gave values indicating correctly the degree of pasteurization if a butyl alcohol extraction is used on the low phosphatase samples.

Results on decomposed samples.—Collaborators 3 and 6 reported the C series of samples as moldy or decomposed. Higher values were obtained when the samples were rerun a few days later. These results are presented in Table 2. It is apparent that cottage cheese samples should be shipped in a frozen condition and kept frozen until analyzed. If decomposition is indicated by organoleptic examination, the samples should be rejected.

Blank readings.—The collaborators were also requested to report the relative magnitude of the blank readings in terms of mmg phenol in the aliquot using the blank corrected phenol curve for the transformation. The magnitude of the blank for U1 varied from 0.5 to 3 mmg in aqueous solution and from 0.5 to 1.5 mmg in butyl alcohol. For C1 the variation was from 3 to 5 mmg in aqueous solution and from 0.5 to 2 mmg in butyl alcohol. A separate blank should be run for each sample if the colors

[3] *This Journal*, 24, 559 (1941).

are to be read in aqueous solution unless it is known that all of the samples in a given series are similar. A single blank for all samples read in butyl alcohol may be sufficient, although the data on this point is not extensive. If a single blank is used for all samples, however, accidental or residual phenolic contamination of a single sample would not be detected.

TABLE 2.—*Phosphatase results on samples reported moldy or decomposed*

COLLABORATOR	DATE ANAL.	C1		C2		C3	
		Bu	Aq	Bu	Aq	Bu	Aq
3	8/5		128		58		11.6
			123*		58		10.2
	8/8		128		96		25.2
			132*		96		24.6
6	8/16	76		27.6		11.5	
		74		31.1		6.9	
	8/28		85		54	20.2	
			97		61	20.2	

* Diluted once.

Recently, Bessey, Lowry, and Brock[4] published a serum phosphatase method using p-nitrophenol phosphate as the substrate. Yellow p-nitrophenol is formed during the hydrolysis which can be measured directly. If this procedure can be applied to dairy products, the phosphatase test for pasteurization can be greatly simplified by the elimination of the color development step of the present method. Since a blank reading can be obtained by merely acidifying the colored solution, difficulties attributable to blanks can be virtually eliminated. This method deserves some study although the present price of the substrate is relatively high.

ACKNOWLEDGMENT

Grateful acknowledgement is due Dr. W. B. Combs and Mr. Al Johnson of the Dairy Department of the University of Minnesota for their assistance in the preparation of the cottage cheese samples, and to the following collaborators (all of the U. S. Food and Drug Administration): Curtis R. Joiner, St. Louis; George McClellan and John F. Weeks, Jr., New Orleans; G. Kirsten, New York; Mrs. Pauline E. Pantsios, Chicago; L. W. Ferris, Buffalo; Charles S. Purcell and Catherine G. Cunningham, Boston.

RECOMMENDATIONS*

It is recommended—

(1) That methods for pasteurization test for soft and hard cheeses be

[4] *J. Biol. Chem.*, 163, 321 (1946).

* For report of Subcommittee C and action of the Association, see *This Journal*, 30, 50 (1947).

brought into agreement so that a single method will suffice for all of these products.

(2) That the method of Bessey, Lowry, and Brock be studied to determine if it can be applied to dairy products.

REPORT ON TESTS FOR RECONSTITUTED MILK

By W. H. King (Chief, Food and Drug Section, Louisiana State Health Department, New Orleans 7, La.), *Associate Referee*

Work on this subject during the current year has been confined principally to compilation and summarizing of previously published information on the subject. Since more references have been found in the literature than were anticipated, this compilation has not been completed.

A limited amount of laboratory work on authentic commercial and laboratory reconstituted milks was done. This work was devoted to comparison of the original Evenson test[1] with two of its modifications.[2,3] Observations under ultraviolet light were also made to test further the possibilities of this diagnostic aid mentioned by King and Schouest.[3]

Seven samples of recombined milks and one authentic laboratory pasteurized milk were tested by the original Evenson test, by two published modifications of the Evenson test, and by fluorescence under the ultraviolet light. The results are tabulated in Table 1.

SUMMARY

The original Evenson test and two published modifications of this test were able to detect three types of 100% reconstituted milk. A mixture of 50% pasteurized fresh milk with 50% milk reconstituted from whole dry milk could be detected by all three methods. The Evenson tests did not show presence of 50% milk reconstituted from plain condensed skim milk and cream mixed with 50% of pasteurized fresh milk. The ultraviolet light did not distinguish between milk reconstituted from plain condensed skim milk with cream, and whole, fresh, pasteurized milk.

CONCLUSIONS

It appears that milk reconstituted from plain condensed skim milk manufactured by present day methods is not always detected by the Evenson test or the ultraviolet test, especially if it is mixed with fresh fluid milk.

RECOMMENDATIONS*

It is recommended that the Evenson test and its modifications be studied further in an effort to make it more sensitive. This would include

[1] *J. Dairy Sci.*, 5, 97 (1922).
[2] (Fairbanks *et al.*), *Ibid.*, 21, 633–6 (1938).
[3] *Quart. Bull., Assoc. Food & Drug Officials*, 8, 136–138 (1944).
* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 50 (1947

TABLE 1.—*Summary of color determinations*

TYPE OF MILK	EVENSON TEST	FAIRBANKS *et al.* MODIFICATION OF EVENSON TEST	KING AND SCHOUEST, MODIFICATION OF EVENSON TEST	APPEARANCE UNDER ULTRA-VIOLET LIGHT
1. Authentic pasteurized fresh milk	White	White	White	Creamy yellow
2. 100% Lab. reconstituted from unsweetened condensed skim milk (bulk commercial grade) and 40% cream and water.	Pale yellow	Pale yellow	Pale yellow	Canary yellow
3. Same as 2. From similar ingredients; different milk plant; different city; commercially reconstituted.	Yellow	Yellow	Yellow	Canary yellow
4. 50% Commercially reconstituted from unsweetened condensed skim milk, cream, water and fresh milk. Different source.	White	White	White	Canary yellow
5. 100% Laboratory reconstituted from canned evaporated milk and water.	Orange yellow	Orange yellow	Orange yellow	Bluish white
6. 100% Laboratory reconstituted from commercial whole milk powder and water.	Pale yellow	Yellow	Pale yellow	Creamy yellow
7. 50% Laboratory reconstituted from commercial whole milk powder, water, and authentic pasteurized milk.	Yellow	Yellow	Yellow	Canary yellow

the Pozzi-Escot[4] Modification, which applies heat to the final color reaction. It is recommended also that a complete review of the literature be made and summarized. Other methods for detection of reconstituted milk should be studied, including a study of the usual chemical and physical constants of different types of reconstituted milk with and without mixture with fresh fluid milk.

ACKNOWLEDGMENT

Thanks are extended to Mr. F. F. Flynn, Chemist in the Chemical-Toxicological Section of the State Health Department, for assistance in the laboratory work.

No general report was made on eggs and egg products, or on added glycerol and salt.

[4] Pozzi-Escot, Emm., *Ann. Chim. Anal., Chim. Appl.*, 5, 272 (1923).

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By J. D. Wildman (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Referee and Associate Referees for extraneous materials in foods and drugs last year rendered reports on their respective subjects and these were published in the February, 1946, issue of the *Journal* of the Association. Since no meeting was held last year no recommendations were made by the Referees. In the work on extraneous materials the attempt has been to provide workable methods for as many as possible of the food and drug products encountered in regulatory and sanitary control work. At the present time we do have available a fairly complete list of methods. Certain new products, however, appear on the market from time to time, and their analysis practically depends on the adaptation of existing methods to the new product. There are also a few broad categories of food and drug products to which relatively little attention has been paid. These are crude drugs, dehydrated foods, and frozen desserts.

The Referee's report will concern itself with those general features of the microanalytical work which would not fall in the respective fields covered by the Associate Referees. In the *Methods of Analysis*, 6th ed., reference is made to the use of a laboratory cyclone having screen openings of ca 0.027″ in diameter. The development of such a cyclone began in 1932 and proceeded by stages until 1944, when a complete laboratory cyclone was furnished to each of the field laboratories in the Food and Drug Administration. The development of the general features of the cyclone followed from experimental operations and conventional design of commercial cyclones. Mr. Albert G. Sterling of the Administration is largely responsible for the mechanical features of the pulper. A description of the cyclone proper follows:

Laboratory Cyclone—The laboratory cyclone consists essentially of a cylindrical screen in which revolves a paddle which forces the soft material out through the openings in the screen. Tough materials such as seeds, skins, and stems are moved along and out of an opening in the end of the cylinder. A one-quarter horse power, 110 volt, electric motor, having a speed of 1725 R.P.M., is used for source of power. The screen is of 22 gauge material having 400 holes per square inch, each .027″ in diameter. The inside diameter of the screen is 2.5″ and the length of effective screen is 3″. The paddle has 2 blades each 25/32″ wide set alternately and extending out $1\frac{3}{16}$″ from center of shaft. In use the pulper is fed through a hopper which leads into a basin $3\frac{1}{2}$″ long and $2\frac{1}{2}$″ inside diameter. A portion of the paddle with fins inserted at a 30° angle forces the material into the screening compartment. The cyclone is so constructed that the waste opening may be closed, as needed. The sieved material is caught in a shield and delivered by means of a spout to a container. The machine may be readily disassembled for washing.

RECOMMENDATIONS*

It is recommended—

(1) That tentative methods described by the Referee and Associate Referees be changed, as recommended by the Referee and Associate Referees in *This Journal*, **29**, p. 54–65 and in Current Reports.

(2) That studies on methods for extraneous matter in foods and drugs be continued.

(3) That the Referee study the nomenclature used in all methods.

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS PRODUCTS

By W. V. Eisenberg (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Since the last report of the Referee on these products,[1] three additional methods have been tentatively adopted and added to this section. These consist of the "Examination of Chicken Giblet Paste for Filth and Sand," by S. H. Perlmutter; "Filth in Canned Fish," by A. H. Wells, and the "Determination of Glass in Meat Scraps," by Chas S. Purcell and W. V. Eisenberg.[2]

The filth in the chicken giblet paste and the canned fish is recovered by the flotation technique using the Wildman trap flask after a preliminary mechanical treatment. The sand in the chicken giblet paste is recovered by sedimentation in chloroform and determined after ignition in a tared crucible.

Glass in meat scraps is recovered by flotation with carbon tetrachloride to decant off the light organic matter, subsequent treatment of the heavy residue with hydrochloric acid to dissolve the bone phosphate and other acid-soluble material which is decanted, leaving a residue of glass and soil particles. The residue is dried and passed through a number of different size sieves and the glass determined by a pick-out under the Greenough microscope of the particles retained on a #40 sieve. The glass present in the finer fractions is determined by a microscopic particle count using the polarizing microscope at a magnification ca 100×.

Since particles of clear quartz may often by mistaken for glass unless examined with polarized light, the Greenough microscope has been fitted with polaroid discs mounted on the stage and in the oculars in such position so as to give the crossed nicols effect. The isotropic or amorphous character of glass is then exhibited, which distinguishes it from crystalline quartz.

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* For report of Committee C and action of the Association, see *This Journal*, **30**, 49 (1947).

[1] *This Journal*, **29**, 56 (1946).

[2] *Ibid.*, **30**, 101 (1947), under "Changes in Methods of Analysis."

No new methods for drugs or spices have been reported. Although only one method is listed for vegetable drugs, analysts have found methods grouped under other products to be suitable for the microanalytical examination of many plant drugs. The spice methods have been found particularly applicable. The method for ground turmeric and cinnamon has especially been recommended for ground barks, roots, and hard fruits.

In choosing a method for a product which is not listed by name in the *Book of Methods*, the analyst should consider its physical and chemical nature rather than its project classification or use. For example, a leafy drug or oily seed may be used as a beverage but a particular spice method may prove most suitable for its examination.

RECOMMENDATIONS*

It is recommended—

That the methods and changes reported in the February, 1946, issue of the *Journal*, and the above-named new methods as published in detail in *This Journal*, **30**, 101 (1947), be adopted as tentative.

It is also recommended that further work be done on the mold count method for capsicum peppers with the object of possibly improving the present technic and securing additional data for the interpretation of results.

No report was given on extraneous materials in dairy products.

REPORT ON EXTRANEOUS MATERIALS IN NUT PRODUCTS AND CONFECTIONERY

By W. G. HELSEL (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Certain changes in the existing methods and one new method were reported in the February, 1946, issue of *This Journal*. Since that time three new methods have been suggested. These are "Filth in Chewing Gum" by Maurice Harris of Houston, K. L. Harris, Washington, and W. W. Wallace, Denver; "Filth in Shredded Coconut" by L. H. Feldstein, Denver; and "Rocks and Decomposed Peanuts in Coarse Peanut Butter" by the Associate Referee. The above-named methods are given in detail in *This Journal*, **30**, 102 (1947) under "Changes in Methods of Analysis."

RECOMMENDATION†

The methods have been used by the analysts of the Food and Drug Administration and found to be satisfactory. It is recommended that the

* For report of Subcommittee C and action of the Association, see *This Journal*, 30, 101 (1947).
† For report of Subcommittee C and action by the Association, see *This Journal*, 30, 50 (1947).

methods and changes reported in the February, 1946, issue of the *Journal* and the three above-named methods, as published in detail in *This Journal*, **30**, 102 (1947), be adopted as tentative.

No report on extraneous materials in canned foods, cereal products, and eggs was given.

REPORT ON EXTRANEOUS MATERIALS IN FRUIT PRODUCTS AND BEVERAGE MATERIALS

By F. ALLEN HODGES (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

A number of new methods for extraneous materials in fruit products have been used by Food and Drug Administration analysts. These methods are listed below. The method for insect and rodent filth in jams and jellies was proposed by Frederick M. Garfield, St. Louis. The method for rot and worm fragments in apple pomace from the manufacture of apple cider was proposed by Dorothy B. Scott, New York, and the method for the detection of caterpillars in Lingon berries was proposed by K. L. Harris, Washington, D. C.

Jams and Jellies, Insect and Rodent Filth.
Apple Pomace from Apple Cider, Rot and Worm Fragments.
Frozen and Canned Blueberries, Rot.
Raisins and Currents, Insect Infestation, Mold, Sand, or Soil,
Lingon Berries, Caterpillars.
Current Pulp, Insect and Rodent Contamination.
Grape Pulp, Rot.
Dried and Canned Fruits (apples, peaches, pears, apricots, plums, and prunes), Filth.

RECOMMENDATIONS*

It is recommended that the changes and methods in the February, 1946, issue of the *Journal* and the above-named new methods and changes as published in detail in *This Journal*, **30**, 103-5, (1947), be adopted as tentative.

REPORT ON EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The following method was found to work well by Earl W. Coulter of

* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 50 (1947).

the Chicago Station of the Food and Drug Administration. It was proposed and recommended by him.

FILTH IN DRIED MUSHROOMS

Maggots. Rapid test for gross contamination. Place 100 g in a beaker and cover with hot H_2O. Boil 30 min. Pour onto a No. 8 sieve in a suitable pan. Add sufficient H_2O to cover the mushrooms. Release the filth by rubbing the mushrooms on the sieve. Filter through a 10XX bolting cloth. Repeat the extraction process as many times as is necessary to recover all the heavy filth.

It is recommended that the above-named method, published in detail in *This Journal*, **30**, 105 (1947), and the changes and new methods for vegetable products given in the February, 1946, issue of the *Journal* of the Association, be adopted as tentative.

No report was given on the general subject of decomposition in foods, including fish products.

REPORT ON GELATIN, DESSERT PREPARATIONS, AND MIXES

By SUMNER C. ROWE (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

It is recommended† that methods for the determination of jelly strength in gelatin be studied, bearing in mind the method of the Edible Gelatin Manufacturers Research Association of America.

No report was made on starch, sugar, and jelly strength.

REPORT ON FISH AND OTHER MARINE PRODUCTS

By ANDREW M. ALLISON (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Referee*

The last Referee report on fish and marine products and subcommittee C action thereon was in 1944 (*This Journal*, **27**, 235; **27**, 62, (1944). Although considerable work has been done on the various phases of the subject under study, the only report since the 1944 meeting of the A.O.A.C. is that of Associate Referee Voth (*This Journal*, **29**, 46) on ether extract in fish.

† For report of Subcommittee C and action of the Association, see *This Journal*, **30**, 51 (1947).

RECOMMENDATIONS*

It is recommended—

(1) That collaborative study be continued on the Modified Rose-Gottlieb Method for determination of ether extract in fish.

(2) That the several methods for determining total solids and ether extract (*This Journal*, **26**, 226–232) be further studied collaboratively with a view to selecting the most suitable method for each constituent.

REPORT ON ETHER EXTRACT IN FISH

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Associate Referee*

A digestion or acid extract method for the determination of fat in fish has been previously described (*This Journal*, **29**, 46, 1946).

During the past year the Associate Referee has done a considerable amount of work on this method. Difficulties encountered by previous collaborators were eliminated. The revised method was then submitted to further collaborative study. An unforeseen minor difficulty arose during this study. The method will therefore not be submitted in its present form until it has been further perfected during the coming year. The Associate Referee wishes to thank the collaborators who participated in this study and regrets that the work cannot be published at this time.

It is recommended†—

That collaborative study be continued on the modified Rose-Gottlieb method for determination of ether extract in fish.

REPORT ON GUMS IN FOODS

By F. LESLIE HART (Food and Drug Administration, Federal Security Agency, Los Angeles, Calif.), *Referee*

No general report on gums in foods can be made; Associate Referees report that studies in their respective assignments have been undertaken which, however, have not developed new methods to a point that would justify recommendations for adoption.

It is recommended‡—

(1) That method for detection of gums in cheese be further studied—

(a) in its quantitative application;

(b) in its application to the detection of soluble alginates.

* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 51 (1947).
† For report of Subcommittee C and action by the Association, see *This Journal*, 30, 49 (1947).
‡ For report of Subcommittee C and action by the Association, see *This Journal*, 30, 49 (1947).

(2) That method for the detection of gums in starchy food, *This Journal*, **29**, 250, be further studied collaboratively.

(3) That studies be continued on the detection of gums in cacao products.

(4) That an Associate Referee be appointed to undertake studies on the detection of gum in pectin-containing food products.

(5) That studies for the detection of gelatin and gums in frozen desserts be continued.

No reports were given on soft curd cheese, mayonnaise and French dressing, frozen desserts, starchy foods, jams, beverage bases, and fruit products, or cacao products.

No general report was given for meats and meat products, or for dried skim milk in meat products and soybean flour in meat products.

REPORT ON SPICES AND CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security A ency, St. Louis, Mo.), *Referee*

Reports were submitted by three Associate Referees.

MUSTARD

Associate Referee Garfield continued his studies on the Whale procedure for determination of starch in mustard. He was unable to eliminate the high and variable blank and consequently abandoned work on this method. He modified the tentative method to minimize loss due to the acidity of prepared mustard, and subjected this method to collaborative study on prepared mustard and on mustard flour. The recoveries averaged 98 per cent on the prepared mustard and 96 per cent on the mustard flour, but some results were as low as 92 per cent. The Associate Referee recommends that the tentative method for prepared mustard be adopted as tentative for mustard flour. The present wording of method **33.42,** under the general heading "Prepared Mustard," describes application to prepared mustard and mustard flour, although successful collaborative work had not previously been done on the flour. It is recommended that the method be modified in accordance with the Associate Referee's suggestions, and be continued as tentative and further studied.

No work was reported on volatile oil in spices, or on ash in prepared mustard. These determinations should be studied next year.

H. A. Lepper has pointed out that the method for copper-reducing substances **(33.40),** which is designed primarily to determine starch, is

inaccurate for that purpose, since it will indicate a certain per cent of starch when none is present (see also Referee's report for 1940). The tentative method for starch, though it may yield slightly low results, does not give a false blank. The method for copper-reducing substances should therefore be dropped, and a method for sugar should be studied. Several procedures are available, among the simplest being the procedure described by Winton in his "Analysis of Foods," p. 900.

MAYONNAISE AND SALAD DRESSING

The Associate Referee submitted the results of collaborative work, which are similar to those obtained previously. The average recovery was 94 per cent. The Associate Referee recommends adoption of the method as tentative. The Referee suggests that with further work on the method the findings reported by Fine (1), that losses in starch occur on aging, be considered.

VINEGAR

Associate Referee Henry submitted a report, J **29,** 304 (1946), covering a study by himself and Mrs. Rokita on the detection and determination of free mineral acids in vinegar. It is recommended that the methods described in his report be adopted as tentative in place of the qualitative indicator methods **33.85** and **33.86,** which are rough tests, and the quantitative titration **(33.87)**, which is in error (2). The present tentative methods should be dropped.

Henry has raised the question (3) whether further collaborative work is justified on the tentative Lichthardt test for caramel, in view of the possibility that the methods described in a recent publication (4) may prove more suitable than the Lichthardt test. It is believed that this method should be studied before collaborative work is continued on the Lichthardt method.

RECOMMENDATIONS*

It is recommended—

(1) That the quantitative method of Mallory and Love for caramel in vinegar be studied before further collaborative work is done on the tentative method for detection of caramel.

(2) That the tentative permanganate oxidation number be studied collaboratively.

(3) That the tentative qualitative **(33.85** and **33.86)** and the quantitative **(33.87)** methods for free mineral acids be dropped.

(4) That the methods for detection and determination of free mineral acids described in the Associate Referee's report (*loc. cit.*) be adopted as tentative.

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* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 53 (1947).

(5) That the official qualitative test for tartrates in vinegar be further studied.

(6) That the method for starch in salad dressings (*This Journal*, **27**, p. 260) be adopted as tentative, and that work on the method be continued.

(7) That the tentative method for starch in prepared mustard be modified in accordance with the improvements described in the Associate Referee's report; that it be made tentative for mustard flour, and that study be continued.

(8) That studies be made of a suitable method for determination of ash in prepared mustard.

(9) That the official method for copper-reducing substances by direct inversion **(33.40)** be dropped, first action.

(10) That studies be made of a suitable method for determination of sugars in prepared mustard.

(11) That studies be continued on volatile oil in spices.

REFERENCES

(1) *This Journal*, **27**, 260 (1944).
(2) *Ibid.*, **28**, 305 (1945).
(3) Private communications.
(4) MALLORY and LOVE, *Ind. Eng. Chem., Anal. Ed.*, **17**, 631 (1945).

No report was given on volatile oil in spices.

REPORT ON STARCH IN PREPARED MUSTARD AND MUSTARD FLOUR

By FREDERICK M. GARFIELD (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In the last report (1) of the Associate Referee two methods were studied for the recovery of added starch in mustard: (a) the Whale method (2) and (b) the Fine mayonnaise procedure (3), which was a modification of Field's (4) adaptation of Sullivan's method for the estimation of starch in plant material (5) to the determination of starch in mustard.

In the Whale procedure the sample was treated with 8 per cent alcoholic potassium hydroxide to remove interfering substances such as oil and proteins. The starch was then taken into solution in 0.7 per cent aqueous potassium hydroxide and recovered and purified by double precipitation with iodine. The Whale method was studied because it shortened the starch determination in prepared mustard, by eliminating moisture and acidity determinations. Recoveries were excellent if a correction were made for a blank on pure mustard. If no correction were made the results were high. During the initial work only single iodine precipitations

were made, although the Whale procedure called for two such purification steps. Since the last report double precipitations were tried, but the blanks continued to remain high. Also they varied with different mustards and with different weights of sample taken for analysis. Apparently interfering substances, subsequently determined as starch, were not removed by the alcoholic potassium hydroxide treatment. This procedure was then dropped.

The blanks obtained, using the revised procedure described,* were low. Both brown and yellow ground mustard seed were examined. Included in the group were authentic California yellow and brown seeds, Romanian (mostly yellow seeds with small amount of brown), Oriental yellow, and Chinese yellow mustard.

The average blank, calculated as starch, was approximately 0.07 per

TABLE 1.—*Starch results on prepared mustard*

TREATMENT	I		II		III		IV	
	NEUTRALIZED WITH N/10 NaOH		NEUTRALIZED WITH $CaCO_3$		UNNEUTRALIZED		BLANKS	
Wt. prepared mustard (g.)	6.2504	5.4132	5.0543	5.4253	4.8484	5.1593	5.0062	5.0513
Wt. starch (100%) added (g.)	0.2165	0.2598	0.2165	0.2598	0.2165	0.2598	—	—
Wt. starch recovered corrected for blank (g.)	0.2181	0.2626	0.2071	0.2556	0.2025	0.2478	0.0060	0.0067
Recovery (%)	100.7	101.1	95.7	98.4	93.5	95.4	—	—

cent with a maximum of 0.2 per cent on Montana yellow mustard. These blanks were definitely within the limits of the accuracy of the method itself. The average blank of 5 commercially prepared mustards (3 salad type and 2 horseradish style) known to be starch free, was approximately 0.05 per cent, with a maximum value of 0.13 per cent.

Fine (3) demonstrated, in his study of the method, that 3–4 per cent of starch was lost in the initial solution of the sample in 30 per cent calcium chloride solution. The calcium chloride solution had been adjusted to 0.01 *N* alkalinity to prevent any possible hydrolysis of starch due to acidity. There was reason to expect further loss in starch if no provision were made to neutralize the free acidity in a prepared mustard before solution in calcium chloride. The following sets of results (Table 1) demonstrate that such losses do occur. Under I the acidity present in the prepared mustard was neutralized with N/1 sodium hydroxide. Under II the acidity was taken care of with calcium carbonate. Under III no acidity adjustment was made, and IV are blanks. All results were corrected for moisture in the prepared mustard.

* The method is shown in detail under "Changes in Methods of Analysis," *This Journal*, 30, 75 (1947).

PREPARATION OF SAMPLES FOR COLLABORATORS

Sample 1—Prepared Mustard

The sample of prepared mustard was a commercial sample to which corn starch was added. The mustard had the following composition:

Ingredients	*lbs.*
Yellow seed	590
Oriental type seed	36
Turmeric	25
Salt	140
White vinegar 100 gr.	1178
Water	2360

The starch used was commercial corn starch which assayed 86.6 per cent starch by direct acid hydrolysis and subsequent determination of dextrose by the Munson-Walker method. Starch was calculated as dextrose×0.9. The prepared mustard mixture was made to contain 5 per cent starch (as is, basis) or 4.33 per cent of 100 per cent starch. Solids and acidity determinations were made by the Associate Referee and were reported to the collaborators.

Acidity—5 g prepared mustard required 2.10 ml of N/1 NaOH for neutralization.

Solids—21.6%.

These figures were to be used in the method and calculations.

Sample 2—Mustard Flour

Difficulty was encountered by the previous Associate Referee and the present one in preparing uniform mixtures of starch and mustard flour. For this reason the starch and mustard were submitted separately. Instructions were to use 2.0000 g of dry mustard flour and 0.2500 g of starch (equivalent to 0.2165 g).

Blanks on both the prepared mustard and mustard flour were negligible.

COMMENTS FROM COLLABORATORS

H. P. Bennett.—"The only difficulty encountered in this analysis was the resuspension of the starch in water after the precipitation with 95% alcohol and centrifuging the first time. Even prolonged shaking failed to break up some of the lumps present."

C. R. Joiner.—"I was unable to get the precipitated starch finely dispersed by shaking. The difficulty in removing the iodine color with the alcohol-NaOH solution appeared to be directly proportional to the size of the starch lumps resulting from inability to disperse them through shaking."

DISCUSSION

The difficulty encountered by both Bennett and Joiner was also encountered by the Associate Referee. This failure to disperse the starch does not seem to give rise to poor results. The wording in the procedure

TABLE 2.—*Results of collaborators*

COLLABORATOR	PREPARED MUSTARD		MUSTARD FLOUR	
	STARCH FOUND	RECOVERY	STARCH FOUND	RECOVERY
	per cent	*per cent*	*grams*	*per cent*
1	4.32	99.8	0.2008	92.7
	4.26	98.3	0.1992	92.0
2	4.03	93.1	0.2009	92.8
	3.95	91.2	0.2016	93.1
3	4.22	97.5	0.2117	97.8
	4.15	95.8	0.2124	98.1
4	4.18	96.5	0.2110	97.5
	4.30	99.3	0.2110	97.5
5	4.54	104.8	0.2088	96.5
	4.66	107.6	0.1992	92.0
6	4.08	94.2	0.2097	96.9
	4.23	97.7	0.2075	95.2
7	4.24	97.9	0.2146	99.1
	4.14	95.6	0.2131	98.4
Average		97.8		95.7

should be changed to read "Stopper and shake the bottle vigorously until the precipitate is as finely dispersed as possible."

The Associate Referee is indebted to the following members of the Food and Drug Administration field laboratory staff: R. L. Felton, Los Angeles, Calif.; H. Van Dame, Cincinnati, Ohio; H. P. Bennett, Kansas City, Mo.; A. W. Hanson, Minneapolis, Minn.; D. Banes, Chicago, Ill.; and C. R. Joiner, St. Louis, Mo.

No work was done on volatile oils in mustard seed.

RECOMMENDATIONS*

In the above method for "Starch In Prepared Mustard and Mustard Flour," it is recommended that the sentence following "Add water to volume of 100 ml" be changed to read "Stopper and shake the bottle vigorously until the precipitate is as finely dispersed as possible."

It is further recommended that the method be adopted as tentative for mustard flour and continued as tentative for prepared mustard and studied further.

* For report of Subcommittee C and action of the Association, see *This Journal*, 30, 53 (1947).

REFERENCES

(1) *This Journal*, **28**, 308 (1945.
(2) *Analyst*, **63**, 328, 441 (1938) and **64**, 588 (1939).
(3) *This Journal*, **27**, 260 (1944).
(4) *Ibid.*, **24**, 700 (1941), and **25**, 705 (1942).
(5) *Ibid.*, **18**, 621 (1935).

REPORT ON STARCH IN MAYONNAISE AND SALAD DRESSING

By DOROTHY M. HELLER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

It was recommended by the Association in 1944 that studies on methods for starch in mayonnaise and salad dressing be continued.

A cooked salad dressing, in which the starch was cooked to a thick paste before adding the salad oil, was prepared to be sent out for collaborative study. The salad dressing contained 6.47 per cent starch (calculated from weight of the air-dried starch added and direct acid hydrolysis value for

TABLE 1.—*Report of starch from salad dressing containing 6.47% potato starch (prepared 2–28–45)*

COLLABORATOR	STARCH FOUND	RECOVERY	DATE ANALYZED
	per cent	*per cent*	
1	6.35	99.1	3- 1-45
	6.36	98.3	
2	6.33	97.8	3-16-45
	6.35	98.1	
3	6.16	95.2	3-14-45
	6.13	94.7	
4	5.89	91.0	3-14-45
	5.98	92.4	
5	6.07	93.8	3-17-45
	6.06	93.7	
6	5.81	89.8	
	5.80	89.6	
7	5.37	83.0*	3-21-45
	5.41	83.6*	
Average		94.4	

* Not included in average.

starch). The starch added was potato starch. The samples were analyzed by the method given in *This Journal*, XXVII (1944), p. 260, using a 3–4 g sample. Results on samples submitted to collaborators are given in Table 1.

The Associate Referee is indebted to the following collaborators, all members of the Food and Drug Administration field laboratory staff: F. Yarnall, H. Van Dame, W. McCarthy, M. E. Warren, G. Keppel, P. Pantsios.

It is recommended* that the method studied[1] be adopted as tentative, and that work on the method be continued.

REPORT ON METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

By H. J. WICHMANN (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Most of the report of the Referee concerns the new organic insecticides containing chlorine in organic combination, but he also makes some comments on the determination of cadmium and copper in foods. No reports on the determination of copper, mercury, or zinc were submitted.

CADMIUM

The report of the Associate Referee on the determination of cadmium demonstrated a flaw not uncovered in the original work on the metal. No recommendation can be made other than that of continued effort to find a method for cadmium that will work well all of the time and not only part of the time.

COPPER

No report on the determination of copper in foods was made by the Associate Referee. However, especial attention is called to a paper published by Greenleaf, the former Associate Referee.[2] Your Referee's interest was aroused by this new method for handling the problem of the adsorption of excess dithizone in those "two-color" dithizone methods for such metals as are best extracted from acid solution. Since dithizone will not strip into the aqueous phase at an acid *p*H, its presence in the chloroform solution will cause an appreciable adsorption even with zero metal and at optimum wave length for the metal dithizonate, but at nonoptimum wave length for dithizone. However, when the constant amount of dithizone, as used in the usual two-color dithizone methods, is almost saturated with metal the absorption of the small excess of dithizone is very small. Therefore, the linear relationship between concentration of metal and absorp-

* For report of Subcommittee C and action of the Association, see *This Journal*, 30, 49 (1947).
[1] *This Journal, loc. cit.*
[2] *This Journal*, 30, 144 (1947).

tion is affected in reverse order by another linear relationship between the absorption of the maximum and the minimum amount of excess dithizone. As a consequence the standardization curve for such metals is linear but less steep than in the "one-color" methods where the excess dithizone is stripped out by the alkaline aqueous solvent. One-color methods have certain disadvantages that cause them to be looked on with disfavor in some instances. By taking advantage of a two-filter system and adding only a slight excess of dithizone in all cases, Greenleaf has developed an all-dithizone method for copper of seemingly high sensitivity and accuracy and without the complication of the absorption of much excess dithizone. The same principle should be applicable to the determination of other metals besides copper, for example zinc, that are best determined by the two-color system from acid solutions. The Referee recommends the Greenleaf paper to the Associate Referees on copper and zinc for further investigation.

"DDT"

The first report of the Associate Referee on the determination of DDT in foods is a survey of methods proposed, and those in actual use. The way is now clear for some competition among the most prominent methods, at least as far as spray residue on fruits is concerned. But the problem does not stop there. The spraying or dusting of DDT on vegetables and forage crops brings other difficulties in sampling, sample preparation, and isolation of DDT. The consumption of foods or feeds sprayed with DDT in quantities greater than the animal body can eliminate causes deposition of the excess DDT in the fatty tissues. Then lactation introduces DDT into the milk because of its solubility in the milk fat. Lactation becomes, in this manner, a second method for the elimination of DDT from the body of the female. The determination of DDT becomes, therefore, of great importance in milk, dairy products, eggs, and in general those foods derived from milk or animal fats. At present we know little or nothing about the contamination of vegetable fats with DDT sprayed on the plants during the growing season, nor about the possibility that plants can translocate DDT or some metabolite from the soil to the tissues of the plant. The determination of DDT, or decomposition products thereof, in the soil, and of their effects on the fertility of soils presents another series of problems. It can be readily seen from the above that the determination of DDT on firm fruits, from which the DDT can be readily stripped with benzene, is the simplest problem we have, and collaboration would better start there. But the Associate Referee will have to go further and pay attention to the problems connected with the sampling, sample preparation, and isolation of DDT from a host of other products. This in itself is no simple task and will mean much careful work. The methods for the final determination of the DDT, while differing greatly in principle, are not numerous, and probably one Associate Referee situated at a strategic

spot will be sufficient to carry the whole burden of directing the collaboration. The Referee recommends that the Associate Referee draw up a specific program as soon as possible and execute it as far as possible by the next meeting.

It has been found that DDT sprayed on fruits does decrease to some extent by volatilization and/or decomposition under the conditions of temperature, sunlight, rain, and wind prevailing in the orchard.[2] The products of decomposition are not well known, but at least some of them can interfere with methods proposed for the determination of residual DDT. Fortunately most of these decomposition products are more volatile than DDT itself and do not seem to accumulate on fruit in the open, but rather disappear about as fast as formed. This is a fortunate circumstance that reduces the difficulties of the DDT determination on such products as grow out in the open. But when canned foods, or soils, are considered other problems arise. The first information[3] is now available on the determination of DDT in canned foods. Apparently there is partial decomposition of DDT in the canning process of some foods, but the mechanism of this decomposition and the nature of the decomposition products are as yet unknown. Naturally, when once formed these products cannot escape from the sealed can. How and to what degree such compounds will affect the analytical methods for DDT have not been studied. The problem will not be simple and solving it may take considerable time and effort. Therefore the Referee recommends the appointment of another Associate Referee to undertake the study of the fate of DDT in the canning process, and the analytical problems involved. It is likely that other problems, concerned with decomposition of DDT where volatilization is restricted or impossible, will arise and call for later attention, but the Referee believes that the Association can cross such bridges when it comes to them.

HEXACHLOROCYCLOHEXANE

The next member of the insecticide family containing organic chlorine that will require attention from the methods point of view is believed to be hexachlorocyclohexane and also known as "B.H.C." (benzene-hexa-chloride) and "666" from its empirical chemical formula, $C_6H_6Cl_6$. The commercial article is made by chlorinating benzene and contains as the principal ingredients four isomers,[4] only one of which, the gamma isomer, appears to have any great insecticidal activity. The toxic properties of these isomers to warm-blooded animals singly or in combination have not been fully determined. The solubility of B.H.C. in organic solvents is about of the same order as that of DDT, and a similar accumulation in the body fat of animals may therefore be expected. Half of the 6 chlorine

[2] *This Journal*, **29**, 218 (1946).
[3] *Ibid.*, **30**, 140 (1947).
[4] A fifth, the epsilon isomers, has recently been found. Commercial B.H.C. also contains small quantities of hepta- and octa- chloro compounds.

atoms are labile to alcoholic potash, and the total chlorine method for DDT is also applicable to B.H.C. Neither method is specific, but determination of the ratio of labile chlorine/total chlorine = 0.5 makes for increased specificity. The only method available (unless the chromatographic method of Ramsey and Patterson[5] for the separation of the isomers can be made into a quantitative method) for determining the gamma isomer in the commercial product employs the infra red spectrometer, but this instrument has not, as far as is known, been used for spray residues. We are working on a colorimetric method for total B.H.C. It is long and complicated and the details have not as yet been fully established. The commercial article has been distributed in 1946 on an experimental basis and, if it follows the DDT pattern, it may be expected to be used on a much larger scale in the 1947 season. It is to be hoped that a practical method for its determination will be available by then.

"OCTA KLOR" OR "1068"

After B.H.C. comes an insecticide which is now known only by the above two trade names. The two manufacturers have agreed on the nonproprietary name "chlordane" suggested by certain government officials. It is said to be an endo-dichloromethylene-dihydro-hexa chlorindane, $C_{10}H_6Cl_8$ in empirical composition, from which comes one of its commercial names. The technical product is a mixture of an uncertain number of isomers and related compounds and exists as a thick, heavy, more or less colored, liquid. Claims for it say it is 3 to 5 times as toxic to certain insects as p,p′ DDT and about as toxic as gamma B.H.C. Little is known about methods of analysis.

"TOXAPHENE" OR "3956"

The last member of the chlorinated family of insecticides called to the attention of the Referee is known by the above trade designations. It is said to be a chlorinated camphene. It is a slightly colored camphor-like solid having a not unpleasant piney odor. Even less is known about its analytical possibilities than its predecessors in the insecticidal field. Whether any of the last three newcomers gain final acceptance in the face of DDT remains to be seen, but the agricultural analytical chemist and the food analyst will have to take them into consideration and do something about them. The Referee calls attention to these new insecticides at this time as indicators of things to come, analytically speaking. Of course, there are other new insecticides that probably have toxic effects on man or other animals, but this family of products, following after DDT, is typical of the post-war era in insecticides and is worthy of the first efforts of this Association in this field. No recommendations for the appointment of more Associate Referees are made since there is, at present,

[5] *Ibid.*, 29, 387 (1946).

nothing to referee among the newcomers. Next year it may be another story.

RECOMMENDATIONS*

(1) It is recommended that methods for the determinations of cadmium, copper, mercury, and zinc in foods be further studied.

(2) That methods for the determination of DDT in foods in general be further studied and that collaboration on the determination of DDT in spray residues on fruits be begun.

REPORT ON CADMIUM

By A. K. KLEIN (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The Associate Referee submitted a method for the determination of cadmium in the range 0–25 mmg in *This Journal*, **28**, 257 (1945). The method as used by him was satisfactory in estimating cadmium in this range with an average error of ±.2 mmg even in the presence of 100 mmg quantities of cobalt, nickel, copper, mercury, bismuth, and zinc, as their soluble salts. The manner of isolating cadmium from the interfering metals was the conventional one; cadmium was finally evaluated colorimetrically as the dithizonate after having been extracted from a 5 per cent sodium hydroxide solution. Fischer and Leopoldi, *Mikrochemie Acta* **1**, 30 (1937), and Sandell, *Ind Eng. Chem., Anal. Ed.*, **11**, 364 (1939), had quantitatively measured cadmium as the dithizonate from a 5 per cent sodium hydroxide solution, and more recently Strafford, *Analyst*, **70**, 232 (1945), has employed this method, although in the last instance 1 *N* sodium hydroxide or approximately 4 per cent was used. So high an alkalinity is used rather than a more favorable lower *p*H value, namely 9.0, because of the possible contamination by zinc. The absorption peak of both zinc and cadmium dithizonate is almost identical, and microgram for microgram, zinc dithizonate has approximately 70 per cent more optical density at its absorption peak than has cadmium dithizonate when developed at a *p*H favorable to both. To avoid this possible source of error, cadmium dithizonate was developed finally in 5 per cent sodium hydroxide, a concentration of alkali in which zinc dithizonate, when not present in too large amounts, does not form, whereas that of cadmium is relatively stable.

The method was submitted for trial to one of the members of the U. S. Food and Drug Administration. The collaborator had no difficulty in preparing standard working curves in the 0–25 mmg range; the curves prepared at daily intervals were superimposable even when different stock solutions of dithizone were used. However, erratic recoveries, always on

* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 49 (1947).

the low side, were obtained when cadmium was taken through the method, even when no interfering metals were present. The Associate Referee, called upon for assistance, also experienced this difficulty, although usually to a much less degree. In his A.O.A.C. 1945 Report, the Associate Referee laid stress on the need for complete purity of dithizone and carbon tetrachloride solvent. As yet no method for testing the purity of the carbon tetrachloride is available and therefore the quality of the solvent used in the first investigation could not be duplicated. Other undiscovered factors warranting consideration may also be involved. However, since preparing the working standard curve offered no difficulty and yet, using the same dithizone carbon tetrachloride reagent, low cadmium results were obtained when the complete method was employed, other factors may warrant consideration. But, because of more urgent problems the Associate Referee was not able to spend further study on the proposed method. However, in the forthcoming year he hopes to uncover the reasons for the vagaries referred to or to devise a better method.

No reports were given on copper, zinc, fluorine, or mercury in foods; see Referee's report, *This Journal*, p. 451.

REPORT ON METHODS FOR THE DETERMINATION OF DDT IN INSECTICIDE RESIDUES AND IN ANIMAL PRODUCTS

By R. H. CARTER (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

Because of the difficulty in preparing and shipping samples, the Associate Referee did not consider it feasible to send out samples for analysis by the collaborators, and so with the consent of the Referee, a survey of the methods in use in various laboratories known to be interested in this subject was undertaken. This report is intended to review briefly the methods which have been found most useful and practical for the determination of DDT residues on fruits, vegetables, forage crops, and forest vegetation, and in animal products such as eggs, fat, meat, milk, and butter.

In general the methods have in common the separation of the DDT residues from the large amount of organic material with which it is associated, followed (1) by conversion of the organically bound chlorine to water-soluble inorganic chlorides and the estimation of the latter by standard methods or (2) by estimation of the DDT by colorimetric methods.

SAMPLE PREPARATION

The first step in preparing the sample is to extract or strip the material with an organic solvent such as benzene, pentane, or petroleum ether. An apparatus similar to the churn-type washer described by Fahey, Cassil, and Rusk (1) is very useful for stripping operations on apples, pears, leafy vegetables, forage crops, and similar materials which are firm enough to withstand tumbling for short periods of time. Extraction by soaking, with occasional shaking, is satisfactory for some types of material; extractions in Soxhlet apparatus may also be employed. Bulky wet materials such as forage crops, leafy vegetables such as cabbage and lettuce, and similar materials must be dried before extraction. Drying at 70° to 80°C. in a circulating air drier has been found very satisfactory. This prevents contamination of the solvent with water and allows a larger sample to be extracted. Care must be taken to prevent entrainment of inorganic chlorides.

Sample preparation for apples and pears and the precautions to be observed have been discussed by Wichmann *et al.* (2).

For animal fats, organs, and flesh samples the following method of preparation has been found satisfactory. A 100-gram sample is macerated in a Waring Blendor with 100 ml. of benzene. This mixture is transferred to an evaporating dish, and the benzene is evaporated on a steam bath. The residue is mixed with sufficient anhydrous sodium sulfate to form a crumbly mass, which is then dried in an oven at approximately 70°C. and again extracted with benzene. Suitable aliquots of the benzene solution are taken, filtered if necessary, and analyzed by chlorine or colorimetric methods.

Milk samples are treated as follows: To samples of 200 grams or more an equal volume of 95 per cent ethyl alcohol is added, and the mixture is extracted with 250 ml of a mixed solvent (75 per cent ethyl ether and 25 per cent Skellysolve B—a hydrocarbon fraction b. p. 60–70°C.) in a separatory funnel. After separation of two layers, the aqueous phase is extracted again with three portions of 100 ml each of the mixed solvent. The solutions containing the DDT and butter fat are then combined and suitable aliquots taken for chlorine or colorimetric determinations. Precautions must be taken here also to prevent entrainment of inorganic chlorides.

METHODS OF ANALYSIS

A review of the chemistry of DDT is given by Cristol and Haller (3) and the chemical composition of technical DDT is described by Haller *et al.* (4).

Inspection of the formula for DDT indicates that there are 5 chlorine atoms, representing 50 per cent of the weight of the molecule. These 5 atoms of chlorine can be converted to chloride ion by treatment with anhydrous isopropyl alcohol and metallic sodium. It is also known that one

of these chlorine atoms, representing 10 per cent of the weight of the molecule, is labile and can be split off by treatment with alcoholic alkali without affecting the other chlorine atoms.

It has also been found that DDT can be treated with various reagents to develop more or less characteristic colors which can be utilized as a measure of the DDT content.

Spectrophotometric methods have also been developed for the estimation of DDT.

Methods for the determination of DDT therefore fall into three classes (1) determination of total chlorine, (2) determination of labile chlorine, and (3) colorimetric and spectrophotometric methods.

It should be remembered that the chlorine determinations are not specific, as other halogen-containing materials may also be present. The ratio of total to labile chlorine may sometimes be used to confirm the presence of DDT.

Colorimetric determinations are not always applicable because of the coloring matter extracted from the plant material. In milk and fat samples the fat is soluble in the solvent, and this complicates analytical procedures. Special methods of preparing samples have been worked out for some materials, and will be discussed in the appropriate sections.

Procedures for the determination of DDT in biological tissues and animal secretions have been described by Smith and Stohlman (5) and Ofner and Calvery (6).

TOTAL-CHLORINE DETERMINATIONS

In the early work on the determination of DDT a modification of the Winter (7) method was employed. This procedure involves the combustion of the organic compound and the absorption of the combustion products in an alkaline sodium arsenite solution, followed by acidification and titration of the chloride ion (8, 9, 10).

However, subsequent experience has shown that a more satisfactory method for the determination of DDT by total chlorine is that of Umhoefer (11), in which organic halogen compounds are decomposed with sodium in refluxing anhydrous isopropyl alcohol. This treatment converts organically bound chlorine into sodium chloride, which may be estimated quantitatively by volumetric or gravimetric procedure. When sulfur compounds are present, sodium sulfide is usually formed during the reduction with sodium. This may be oxidized to sulfate by boiling with alkaline hydrogen peroxide before acidification and titration for chloride. This preliminary oxidation is often useful in eliminating colored materials which interfere with the chloride titration. Colored solutions may sometimes be cleared up by extraction with an organic solvent immiscible with water or by the addition of chlorine-free charcoal and subsequent filtration. Determination of "blank" values on untreated materials from the

same source is of extreme importance, especially when only small amounts of DDT residues are present.

Procedures for the determination of total organic chlorine in residues from DDT deposits on agricultural crops are described by Wichmann *et al.* (2) and Carter and Hubanks (12).

This method with suitable modifications has been used in our laboratories for determining organic chlorine residues in a wide variety of products, such as apples, pears, peaches, quinces, potatoes, tomatoes, cabbage, lettuce, forage crops, milk, eggs, animal fats, and tissues. Modifications necessary in the determination of organic chlorine in milk are described by Carter (13). The determination of total chlorine as a measure of DDT residues has been reported as being used in a number of other laboratories.

LABILE-CHLORINE DETERMINATIONS

The reaction between DDT and ethanolic alkali forms the basis for a method of determining DDT which has been developed and described independently by Gunther (14) and by Neal and coworkers (15). The concentrated organic halogen-containing materials are treated with ethanolic sodium or potassium hydroxide and heated at reflux to dehydrochlorinate the DDT, then cooled, acidified, and the hydrolyzed chloride-ion content measured by one of the usual methods. As this method measures only 1 of the 5 chlorine atoms in the DDT molecule, the other 4 being stable to the treatment, analysis by this method is correspondingly less sensitive than the total chlorine methods. This is important, since the tentative tolerance of 7 p.p.m. which has been established for DDT on apples is equivalent to only 3.5 mg. of total chlorine per kilogram of material and to only 0.7 mg of hydrolyzable chlorine.

This method is considerably more specific than the total chlorine determination, since organic chlorine compounds which are not hydrolyzed by ethanolic alkali do not interfere. Such DDT degradation products are 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, bis(*p*-chlorophenyl)acetic acid, and *p,p'*-dichlorobenzophenone do not have labile chlorine atoms and thus do not interfere. Isomers of DDT, some byproducts in technical DDT, and benzene hexachloride are typical interfering materials which do have labile chlorine atoms. The ratio of labile to total chlorine may sometimes be used as an indication of the presence of DDT.

Descriptions of the methods and comparison of the results obtained from determinations of total and labile chlorine resulting from DDT deposits on agricultural crops is discussed by Carter and Hubanks (12). Details of this method applicable to residues on apples and pears have been described by Wichmann *et al.* (2).

The final determination of the chloride ion may be made by any standard procedure. In our laboratories we have found titration with silver nitrate, using an electrometric titrimeter, to be very satisfactory.

The labile chlorine method has been reported as being in use in a number of laboratories. It is the consensus of opinion that normal residues of DDT give the theoretical ratio between labile and total organic chlorine within experimental error when both methods are carried out with due precautions.

COLORIMETRIC DETERMINATIONS

Schechter-Haller colorimetric method.—One of the most sensitive and specific quantitative tests for DDT is the color test developed in the Bureau of Entomology and Plant Quarantine (Schechter *et al.* (16)). In this procedure the DDT-containing concentrate is treated with a mixture of nitric acid and sulfuric acid, which converts DDT to the tetranitro derivative. This derivative when treated with methanolic sodium methoxide gives rise to a fairly stable blue color, which has been shown to follow Beer's law and thus may be used in an estimation of DDT. The spectrum of this material has a maximum absorption at 600 millimicrons, and the amount of DDT may be determined photometrically. As little as 10 micrograms of DDT may be determined with reasonable accuracy by this method. The method is relatively free of specific interfering compounds, with the exception of the *o,p'*-isomer of DDT and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethane (sometimes called TDE or DDD). The latter gives an almost identical spectrum and thus cannot be differentiated from DDT, while the former gives a sufficiently different spectrum to allow analysis for the *p,p'*- and *o,p'*-isomers in the presence of each other. The degradation products of DDT mentioned under Labile-Chlorine Determinations do not interfere in the test, since the red colors produced by these substances are spectroscopically different from the blue color produced by DDT. In effect, then, production of the typical spectrum from a DDT deposit provides excellent evidence for the presence of DDT in the deposit, and such a determination should be made whenever there is a reasonable possibility that total chlorine or hydrolyzable chlorine determinations may not be valid. The color method does not lend itself to analysis of large numbers of samples so readily as the other methods, since more steps are involved. In addition, it is necessary to have available a spectrophotometer for color measurements.

Application of this method to the determination of DDT in spray residues on fresh fruit is discussed by Wichmann *et al.* (2).

A modification of this colorimetric method which is suitable for the determination of DDT in fatty materials such as milk, butter, meat, and eggs is described by Schechter *et al.* (17). The preparation of the sample consists in washing a chloroform solution of butter fat and DDT with one or more portions of sodium sulfate-sulfuric acid solution and then with fuming sulfuric-concentrated sulfuric acid mixture and a final portion of sodium sulfate-sulfuric acid solution. After suitable separation and neutralization of the solutions and evaporation of the solvent, the residue is

nitrated and the determination carried out as originally described (16).

Close agreement between the total chlorine method and this modification of the colorimetric method for DDT in milk samples has been obtained in our laboratory. We have also used this modification on colored extracts of vegetables, forage crops, and similar materials, with very good results.

Dinitrophenylhydrazine method.—This method, described by Wichmann *et al.* (2), is based upon three separate chemical reactions—namely, the conversion of *p,p'*- and *o,p'*-DDT to the corresponding ethylenes, oxidation of the ethylenes to the dichlorobenzophenones, and the conversion of the dichlorobenzophenones to the 2,4-dinitrophenylhydrazines. The last derivatives yield colors in alkali which are qualitatively identical but which differ quantitatively by about 10 per cent, the *p,p'*-isomer giving the higher value.

The authors conclude that this method will probably never be used as a routine method but will be extremely useful as a check on other methods.

Xanthydrol-pyridine-potassium hydroxide method.—This method is described by Stiff and Castillo (18, 19, 20) as specific for DDT. It makes use of a xanthydrol-pyridine-potassium hydroxide reagent, which yields a red color. These authors give directions also for making field tests for surface deposits of DDT.

This reaction has been investigated by Claborn (21), who gives a procedure for the quantitative determination of DDT in apple spray residues and a proposed qualitative test for DDT on apples.

This method appears to have promise for the rapid determination of DDT residues on apples and pears. It may also be applicable to the determination of DDT in milk and meat products. Its application to leafy vegetables, fruits, forage crops, and forest vegetation, where other coloring matter may also be extracted, has not been worked out.

Chaikin (22) has recommended a colorimetric method for the determination of *p,p'*-DDT applicable to amounts ranging from 50 to 500 micrograms. It is based on the color produced when this compound is heated in a mixture of glacial acetic and concentrated sulfuric acids. The method has not been applied to insecticidal residues of DDT.

Bailes and Payne (23) have described a method based on the application of the Friedel-Crafts reaction, which gives a stable compound that is orange in transmitted light and greenish orange in reflected light.

Illing and Stephenson (24) have presented two colorimetric methods for the detection and estimation of small amounts of DDT. The first method is based on the nitration of DDT and treatment of the nitrated product with ammonia and hydroxylamine hydrochloride to develop a fluorescent orange color. The second method was suggested by the fact that the tetranitro compound of DDT gives a purple color with alcoholic potash in the cold. Applications to the determination of DDT in residues from insecticidal applications have not been reported.

Several other colorimetric methods have been reported but they have not been applied in residue determinations.

A spectrophotometric method has been proposed by Herriott (25) by which 10 to 50 micrograms of *p,p'*-DDT may be determined with an error not greater than 10 per cent. When dissolved in 95 per cent ethanol, *p,p'*-DDT absorbs light very slightly at 250 millimicrons. After dehydrochlorination by dilute alcoholic sodium hydroxide to the olefin, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, the solution absorbs strongly at this wave length. Measurement of this increase in absorption and comparison with that obtained with a similarly treated standard DDT solution is the basis of this method.

Downing *et al.* (26) discussed the application of infra-red spectroscopy to the quantitative determinations of DDT, including insecticidal residues.

SUMMARY AND CONCLUSIONS

Methods for the determination of DDT in insecticidal residues on a wide variety of materials by determinations of total and labile chlorine have been described. In general the agreement between these two methods is within the limits of experimental error.

Several colorimetric methods for the detection and estimation of DDT have been described. These methods have given results substantially in agreement with those obtained by total and labile chlorine methods. Wichmann *et al.* (27) conclude that decomposition products of DDT can be produced but that apparently their volatile nature assures their removal under ordinary circumstances about as fast as they are formed, and that apples exposed freely to sun, rain, and wind of the orchards may be expected to be free of them.

It appears, therefore, that reliable determinations of DDT residues may be obtained by total and labile chlorine determinations, provided other halogen-containing compounds are absent. Some of the colorimetric methods are applicable if the residue extracts can be purified and decolorized sufficiently to allow the characteristic colors to be developed.

The Associate Referee recommends that studies to compare the results obtained by the different methods be continued and that shorter, simpler, confirmatory qualitative and quantitative tests be developed if possible.

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REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

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

RECOMMENDATIONS*

It is recommended—

(1) That the study of methods for determining physostigmine in ointments be continued and reassigned.

(2) That the method submitted by the Associate Referee for quinine ethyl carbonate be adopted as tentative and the subject closed.

(3) That the investigation on the separation of theobromine and phenobarbital be continued.

(4) That the tentative method for the determination of prostigmine be adopted as official, first action, and the subject closed.

(5) That the study of the separation of aminopyrine, ephedrine, and phenobarbital be continued.

* For report of Subcommittee B and action by the Association, see *This Journal*, **30**, 45 (1947).

(6) That the work on ephedrine be continued and the method reported by the Associate Referee be submitted for collaborative study.

(7) That the study of methods for the determination of camphor in spirits of camphor be continued.

(8) That a study be made on chemical methods for the estimation of penicillin.

(9) That studies be continued on chemical methods for ergot alkaloids.

(10) That studies on methods for determination of quinine be continued.

No report was given on chemical methods for ergot alkaloids, or on physostigmine in ointments.

REPORT ON QUININE ETHYLCARBONATE IN TABLET MIXTURES

By M. DRUCKER (U. S. Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

A study was undertaken to outline a method for determining quinine ethylcarbonate in a tablet mixture.

Previously, H. G. Underwood[1] reported that hydrolysis, followed by extraction and titration of the resulting quinine, was a satisfactory procedure. However, it was suggested that this method could be simplified by eliminating the hydrolysis and subsequent extraction by titrating the quinine ethylcarbonate directly with perchloric acid in glacial acetic acid. While this is a general method applicable to all weak bases, it has the disadvantages of requiring a special indicator, of rendering and keeping the perchloric and acetic acids anhydrous, and of preparing a standard perchloric acid solution which would be needed infrequently in some laboratories.

With the above objections in mind, it was decided to adapt the first method to a quinine ethylcarbonate tablet mixture. The collaborative sample was prepared from tested quinine ethylcarbonate U.S.P. XII, potato starch and lactose, theoretically containing 30.2 per cent anhydrous quinine.

The method was subjected to collaborative study by members of the Food and Drug Administration; results are shown in Table 1.

Details of the method are given under "Changes in Methods of Analysis," *This Journal*, **30**, 86 (1947).

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[1] *This Journal*, 25, 824–828 (1942).

TABLE 1.—*Anhydrous quinine in tablet mixture*

COLLABORATOR	GRAVIMETRICALLY		VOLUMETRICALLY	
	per cent found	*per cent of theoretical*	*per cent found*	*per cent of theoretical*
J. P. Aumer,	29.7	98.3	29.7	98.3
New Orleans	29.7	98.3	29.6	98.0
A. L. Diamond,	31.7	105.0	31.6	104.6
New York	31.8	105.3	31.8	105.3
F. M. Garfield,	30.2	100.0	28.5	94.4
St. Louis	30.2	100.0	28.8	95.4
	30.1	99.7	28.6	94.7
R. Goldman,	31.0	102.6	29.6	98.0
New York				
M. Matluck,	31.6	104.6	29.9	99.0
Boston	31.1	103.0	29.9	99.0
	30.4	100.7	30.4	100.7
	31.0	102.6	30.0	99.3
R. D. Stanley,	30.1	99.7	29.3	97.0
Chicago	30.0	99.3	29.9	99.0
			29.3	97.0
Associate Referee	30.3	100.3	30.0	99.3
	30.0	99.3	29.5	97.7
	30.2	100.0	30.5	101.0
	30.4	100.7	30.3	100.3
Per Cent Average Recovery		101.1		98.8

COLLABORATORS' COMMENTS

J. P. Aumer.—No difficulty was experienced in following the method.

F. M. Garfield.—The residue weighed after the extraction was brown in color and the subsequent solution was a cloudy yellow. The color in the solution made titration somewhat difficult.

M. Matluck.—Trouble was encountered in picking the correct end point. The "yellow color" end point specified in **39.13** could only be attained with a considerable excess of 0.02 *N* acid. The actual color, at the correct end point of *p*H 5.5–6, was found to be a yellow green. This color was attained so gradually that the correct stopping point was difficult to judge and could easily be missed by 0.1 or 0.2 ml.

DISCUSSION

The collaborative study indicates that both the gravimetric and the volumetric determinations give equally satisfactory results.

The Associate Referee suggests that the addition of 25 ml of water to

the alcoholic solution of the dried alkaloid before titrating will produce a "yellow color" closer to the true end point than the A.O.A.C. procedure.

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

No report was made on theobromine and phenobarbital.

REPORT ON PROSTIGMINE

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

The method for the determination of the salts of prostigmine was studied last year and reported (*This Journal*, **28**, 686). The method is based on the hot alkaline hydrolysis of the prostigmine salts with the formation of dimethylamine which can be subsequently distilled and collected in a measured amount of standard acid. The details of the method appear in *Methods of Analysis, A.O.A.C.*, 6th Edition, 1945, p. 693.

In accordance with the recommendation of Subcommittee B, the Associate Referee prepared an authentic mixture consisting of 7 per cent prostigmine bromide, 73 per cent lactose, and 20 per cent starch, and sub-

REPORT OF COLLABORATORS

Collaborator	*Prostigmine Bromide* *Per Cent*
1	7.01
	6.93
2	6.52
	6.71
	6.73
3	6.82
	6.79
4	6.71
	6.73
5	6.71
	6.69
Associate Referee	6.55
	6.54
Average	6.73

* For report of Subcommittee B and action by the Association, see *This Journal*, 30, 45 (1947).

mitted samples to collaborators for the purpose of determining the accuracy of the method. Assay of the prostigmine bromide used in this mixture indicated a purity of 99.5 per cent. The theoretical amount of prostigmine bromide in the prepared mixture would therefore be 6.97 per cent.

The Associate Referee wishes to express his appreciation to the following collaborators, all members of the Food and Drug Administration:

W. H. Munday, Kansas City, Mo.; Daniel Banes, Chicago, Ill.; Dona S. Clark, and Gloria Getchell, Minneapolis, Minn.; Sam Fine, Cincinnati, Ohio.

DISCUSSION

The average results of six analysts of 6.73 per cent prostigmine bromide is 96.6 per cent of the theoretical amount of the salt in the prepared sample. Blanks reported by the various collaborators show a variation from 0.28 to 1.08 ml of N/50 acid. It is hard to understand why there should be such a wide variation in blanks between collaborators. It is apparent that this difference in blanks is in a large measure responsible for the spread between analysts in the amount of prostigmine bromide reported.

It is recommended* that the tentative method for prostigmine be made official, first action, and the subject closed.

No reports were given on aminopyrine and phenobarbital, or on quinine.

REPORT ON THE DETERMINATION OF EPHEDRINE

By LLEWELLYN H. WELSH (Chemical Section, Medical Division, U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

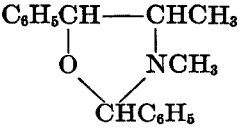
The current edition of *Methods of Analysis* (1) describes methods (Official, First Action) for the determination of ephedrine in inhalants and tablets. In these methods the alkaloid is ultimately determined by extracting it into ether from an ammoniacal solution, evaporation of the ethereal extract to a small volume in order to drive off ammonia, addition of excess standard acid, and back-titration with alkali after removal of the remainder of the ether. Dissatisfaction with the methods apparently has been expressed in several quarters, and, at the suggestion of the Chairman of Subcommittee B, a study of the determination of the drug in its various dosage forms was undertaken by the Associate Referee.

Rather than attempt to investigate the procedures in *Methods of Analysis* with the objective of locating and remedying the causes of the diffi-

* For report of Subcommittee B and action by the Association, see *This Journal*, 30, 45 (1947).

culties, it was decided to abandon the procedures entirely and investigate other possibilities. Even though modifications might be found which would yield consistent and acceptable recovery figures, the methods would still merely measure a base, soluble in organic solvents, and would not yield any information as to the identity and purity of the substance measured. The same is true of assays for ephedrine preparations in the U. S. P. (2) and N. F. (3). Methods have been described (4, 5, 6) for the determination of ephedrine by distilling it with steam and titrating the distillate. Such methods would eliminate any nonvolatile bases being determined as ephedrine, but the results would be affected by ammonia or other volatile bases. Although it might be possible to devise an analytical procedure which would determine ephedrine only in the presence of substances of similar properties, such a method undoubtedly would be quite complicated. A practical alternative, and one of considerably more value than those appearing in the official compendia, would be a direct method in which the alkaloid is isolated and determined as such, or in the form of a derivative the properties of which can be investigated by the analyst for assurance that the substance measured is the substance sought. Among drugs which are commonly determined quantitatively by such a procedure are caffeine, acetophenetedin, and many alkaloids. The method described by Jackerott (7) for the determination of ephedrine in oils seemed to satisfy the requirements. In the assay, ephedrine is extracted from the oil into acid, which is then basified, and the ephedrine is extracted into chloroform. Since ephedrine is too volatile to isolate and determine as the base, it is quantitatively converted to the hydrochloride by floating a few drops of concentrated hydrochloric acid on the chloroform extract, and removing the solvent on the steam bath in a current of air. Although it is well known that chloroform reacts with ephedrine, under suitable conditions, to form ephedrine hydrochloride and benzaldehyde, the known reaction products, it is evident from work in this laboratory that under the assay conditions no more than very slight decomposition of ephedrine occurs, and the use of chloroform, which has such desirable physical properties, appears fully justified.

The assay procedure proposed is essentially that of Jackerott (7) with some modifications. Oily inhalants and nasal jellies of a petroleum jelly base containing only free ephedrine require no preliminary treatment, and the first step is an acid extraction of a benzene dilution of the oil or jelly. Some oily inhalants on the market, however, contain little, if any, free ephedrine, and, instead, contain compounds known as oxazolidines, which are prepared by reacting ephedrine, or other β-amino alcohols, with aldehydes or ketones (8). An example is 3,4-dimethyl-2,5-diphenyloxazolidine (9) (erroneously termed "benzalephedrine") (10) which results from benzaldehyde and ephedrine.



Such compounds are feebly basic, usually are quite stable, and frequently are crystalline substances which are readily and quantitatively cleaved by hot dilute acids to the parent amino alcohol and carbonyl compound. Oils containing these substances are first boiled for ten minutes with 5 per cent acid. Water-soluble jellies and syrups must first be diluted with water to form a suitably thin fluid from which, after basifying with sodium hydroxide, the ephedrine is extracted into ether.

PRELIMINARY EXPERIMENTAL WORK

A mixture of 0.1000 g of *l*-ephedrine hydrochloride, 20 ml of water, and 1 ml of 20 per cent sodium hydroxide, was extracted with five 20 ml portions of benzene. The benzene extract was filtered through cotton into a tared beaker, an excess of an ethereal solution of hydrogen chloride was added, and the mixture was evaporated to dryness on the steam bath in a current of air. The residue of ephedrine hydrochloride, dried at 110°C., weighed 0.1003 g.

When five 15 ml portions of chloroform were substituted for benzene in the above procedure, a residue of 0.1003 g was again obtained. When, in addition, five drops of concentrated hydrochloric acid was substituted for the ethereal hydrogen chloride, the residue weighed 0.0999 g.

These results indicate that any loss of ephedrine due to decomposition by chloroform is negligible, and that the hydrochloric acid converts the base quantitatively to the hydrochloride without loss from volatilization.

PROPOSED METHOD

If the product is an oily solution of ephedrine chemically combined with carbonyl compounds, choose a sample which will contain 20–100 mg of ephedrine. Transfer it to a 125 ml Erlenmeyer flask with 5 ml of benzene, add 10 ml of 5% sulfuric acid, and agitate frequently and swirl the mixture while it is boiled for ten minutes on a hot plate. After cooling, transfer contents of flask to a separatory funnel, and rinse the Erlenmeyer with portions of benzene totalling about 15 ml in order to remove all oily matter from it. (It is desirable that the flask be provided with a lip in order to facilitate the quantitative transfer of the contents to the funnel.) Shake the funnel containing the acid and benzene rinsings,[1] drain off the acid layer into a second separatory funnel, and extract the benzene-oil phase with three 5 ml portions of water with which the flask has been previously rinsed. Wash the aqueous solution of ephedrine sulfate with one or, if considered necessary, two 3 ml portions of chloroform, basify with ca 2.5 ml of 20% sodium hydroxide, and extract the ephedrine with four 15 ml portions of chloroform. If more than 50 mg of ephedrine

[1] In the transfer of ephedrine from organic solvent to aqueous phase, and vice versa, shake-outs should e conducted for one full minute.

is present, filter the extracts through a pledget of cotton into a tared 100 ml beaker, previously dried at 110° and cooled in a desiccator, rinse the cotton with chloroform, and add five drops (0.2 ml) of concentrated hydrochloric acid to the filtered extracts and washings. Heat the beaker on the steam bath in a current of air until the volume of the contents has been reduced to about 1 or 2 ml. Then cautiously heat it, without the air current, until the odor of hydrogen chloride has disappeared and the residue is apparently dry. Heat the beaker in an oven at 110° for one-half hour, cool in a desiccator, and weigh. If less than 50 mg of ephedrine is present, evaporate the filtered extracts in a 100 ml beaker, as described above, until the chloroform, but not the excess hydrochloric acid, has been removed. Direct around the inside of the

TABLE 1

PRODUCT ANALYZED	APPROX. SAMPLE SIZE	EPHEDRINE BASE		PER CENT RECOVERY	CORR. M. P. OF RESIDUE °C.
		PRESENT OR THE EQUIVALENT	FOUND		
Aqueous solution ephedrine hydrochloride	10 ml	0.8192 g/100 ml	0.810 0.804	98.9 98.1	—
Jelly Ephedrine Sulfate, N. F. VII	10 g	0.7711%	0.7740 0.7559	100.4 98.0	216 –217.5°
Syrup Ephedrine Sulfate, N. F. VII	10 ml	0.3078 g/100 ml	0.313 0.308	101.7 100.1	216.5–218°
Ephedrine Inhalant Comp., N. F. VII (plus 0.2% methyl salicylate)	10 ml	1.000 g/100 ml	0.988 0.984	98.8 98.4	216.5–217.5°
Light liquid petrolatum soln. of 3,4-dimethyl-2,5-diphenyloxazolidine	10 ml	1.000 g/100 ml	0.989 0.981 0.984	98.9 98.1 98.4	216 –217.5°
Average				99.1	

beaker a fine stream of redistilled reagent grade methanol to dissolve the ephedrine salt, and immediately repeat the process with a stream of chloroform. Transfer the methanol-chloroform solution to a tared 20 ml beaker, previously dried at 110° and cooled in a desiccator, and repeat the methanol and chloroform rinsings until the ephedrine hydrochloride has been quantitatively transferred. Evaporate the solution on the steam bath, in a current of air, until the salt begins to crystallize. Continue the removal of solvent by cautious heating, to avoid loss from crepitation, until the residue is apparently dry and there is no odor of hydrogen chloride. Dry the residue as previously described.

If the product is an oil containing free ephedrine, transfer it to a separatory funnel with 15 ml of benzene, extract the mixture with 5 ml of 10% sulfuric acid, then with four 5 ml portions of water, and complete the assay as above. In the assaying of petroleum jelly preparations, dissolve the sample in enough benzene to obtain a solution of suitable fluidity (30 ml should be sufficient for a 10 g sample).

If the product consists of a water-soluble jelly, or syrup, transfer it to a separatory funnel with enough water to bring the volume to 20 ml, make the mixture alkaline with the minimum amount of 20% sodium hydroxide, and extract with five 20–25 ml portions of ether. Then extract the ethereal solution of ephedrine with 5 ml of 10% sulfuric acid and four 5 ml portions of water, and continue the assay

as described for oily products. With thin jellies, such as the N.F. VII preparation, samples up to 10 g may be used, but with thick jellies the sample size must be reduced sufficiently to avoid the formation of emulsions. Simple solutions of ephedrine salts need not be diluted to 20 ml before basifying and extracting with ether.

TABLE 2

PRODUCT ANALYZED	APPROX. SAMPLE SIZE	EPHEDRINE BASE		PER CENT OF DECL.	CORR. m. p. OF RESIDUE °C.
		DECLARED OR CALCULATED FROM DECLARATION	FOUND		
Inhalant,[a] "plain," manufacturer A, lot 1	10 ml	1.000 g/100 ml	1.048 1.041	104.8 104.1	215.5–217°
Inhalant,[a] "plain," manufacturer A, lot 2	10 ml	1.000 g/100 ml	1.022 1.026	102.2 102.6	216 –217.5°
Inhalant,[b] "compound," manufacturer A	10 ml	1.000 g/100 ml	0.992 0.999	99.2 99.9	216.5–217.5°
Inhalant,[c] manufacturer B	10 ml	0.7711 g/100 ml	0.752 0.768	97.5 99.6	216.5–218°
Inhalant,[d] manufacturer C	10 ml	0.934 g/100 ml*	0.918 0.915	98.3 98.0	217.5–218°
Nasal jelly,[e] manufacturer B	10 g	no quantitative declaration	0.371% 0.357% 0.366%	— — —	215 –217°
Nasal jelly,[f] manufacturer D	10 g 5 g 5 g	0.4915%	0.494 0.504 0.498	100.5 102.5 101.3	215.5–218°
Syrup,[g] manufacturer E	10 ml	0.1690 g/100 ml	0.168	99.4	—
Syrup,[h] manufacturer A	10 ml	0.1690 g/100 ml	0.169 0.168	100.0 99.4	216 –218°

[a] A light liquid petrolatum base, aromatized, containing ephedrine combined with cinnamic aldehyde and benzaldehyde.
[b] A light liquid petrolatum base containing ephedrine, oil of thyme, menthol, and camphor.
[c] An isotonic, aqueous solution containing ephedrine sulfate, sodium chloride, chlorobutanol 1.36 grains/fl. oz., and benzyl alcohol 1.92 grains/fl. oz.
[d] An isotonic, aqueous solution containing ephedrine lactate, chloretone 0.5%, glucose, menthol, and aromatics.
[e] A petroleum jelly base containing ephedrine, camphor, menthol, and cineole.
[f] A water-soluble jelly base containing ephedrine hydrochloride, chlorobutanol 0.5%, sodium chloride, menthol, and cinnamon oil.
[g] A syrup containing ephedrine sulfate, sugars, flavoring and coloring agents, and alcohol 12%.
[h] A syrup containing ephedrine sulfate, glucose, tolu, vanillin, benzaldehyde, amaranth, and alcohol 12%.
* This figure was obtained by applying the U.S.P. XII assay for ephedrine sulfate. The label actually declared the equivalent of 1% ephedrine base.

DISCUSSION

Tables 1 and 2, respectively, list results obtained on preparations compounded in this laboratory and on preparations of various pharmaceutical houses. Ephedrine hydrochloride residues ranged in weight from 20 to 120 mg, and in color from almost white to light tan. Material from the N.F. VII syrup was the most highly colored. Temperatures of initial fu-

sion of the residues were 3–4 degrees lower than the 219–219.5° melting range of recrystallized ephedrine hydrochloride, but in only three cases was it lower than the 216° minimum allowed by the U.S.P. XII, and in these cases the difference was no more than one degree.

Difficulty with emulsions was encountered only in assaying the 10 g sample of nasal jelly, manufacturer D, at the point of extracting the diluted alkaline jelly with ether. When the sample size was reduced to 5 g no trouble was experienced. Benzene did not prove an acceptable substitute for ether in extracting alkaline solutions of syrups and water-soluble jellies because of its tendency to form emulsions.

In assaying the petroleum base nasal jelly of manufacturer B, it was necessary to dissolve the sample in 30 ml of benzene in order to obtain a solution of suitable fluidity.

A 10 ml volumetric flask was used to measure out samples of oils and syrups, the nature of which precluded the use of a pipet.

When the preliminary 10-minute boiling with dilute acid was omitted in assaying a 10 ml sample of "plain" inhalant, manufacturer A, the residue ultimately obtained consisted of 0.144 g of a brown resin. When dissolved in a mixture of methanol and a small amount of hydrochloric acid, and heated on the steam bath, odors of benzaldehyde and cinnamic adlehyde were observed. Finally, there was obtained a residue of ephedrine hydrochloride contaminated with a light-brown resinous material.

Observations in this laboratory suggest that the rate of reaction between ephedrine and polyhalomethanes increases with the concentration of ephedrine. In order to minimize any loss due to the occurrence of the reaction in films formed as a result of solvent evaporation, the filter funnel and the tip of the separatory funnel were rinsed with chloroform after draining off and filtering each extract. It is not desirable that the assay be unduly delayed while the ephedrine is in chloroform solution.

Methanol alone is not used in transferring residues of ephedrine hydrochloride because the solvent has a tendency to creep, absorb moisture as it evaporates, and to complicate the transfer. The joint use of chloroform and methanol as described practically eliminates these difficulties.

The proposed method is more time consuming and laborious than ones currently official, but it is considered that the advantages more than compensate for the extra time and labor. It has not been applied, as yet, to tablets or capsules of ephedrine salts, but it is not believed that such dosage forms should present any problems. Good recoveries should be obtained from preparations containing racemic ephedrine, since this substance, unlike the optically active forms, is sparingly soluble in water.

RECOMMENDATIONS*

It is recommended that the subject be continued and that the proposed method be studied collaboratively.

* For report of Subcommittee B and action by the Association, see *This Journal*, 30, 45 (1947).

REFERENCES

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(8) STUART, E. H., (to Eli Lilly and Co.) U. S. Patents 1,749,361 and 1,749,452 (Mar. 4, 1930).
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No report was given on spirit of camphor.

REPORT ON SYNTHETIC DRUGS

By L. E. WARREN* (Drug Division, Chemical Section, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Fourteen topics were assigned this biennium. Of these, only seven Associate Referees submitted any reports and two of these were in the nature of progress reports. The Referee is recommending that methods for five substances be adopted as tentative. He further recommends that three topics be discontinued and that seven topics be continued. The Referee is recommending that six new topics be studied.†

No reports were received on the following topics.

Phenothiazine	Carbromal
Demerol	Butacaine Sulfate
Sulfanilamide Derivatives	Spectrophotometric Methods

Butacaine Sulfate.—No report was received. It is recommended that the subject be continued.

Spectrophotometric Methods.—No report was received. It is recommended that the topic be continued.

Plasmochine.—A report was submitted at the meeting. The Associate Referee recommended that a method which he and his associates had developed be adopted as tentative and that the subject be closed.

Metrazole.—A progress report has been published (*This Journal*, **29**, 296, 1946). Additional collaborative work was done. The results were moderately good. The Associate Referee recommends that the method be adopted as tentative and that the topic be closed.

The Referee concurs.

* Present address: 2 East Raymond St., Chevy Chase, Md.
† For report of Subcommittee B and action by the Association, see *This Journal*, **30**, 46 (1947).

8-Hydroxy-quinoline Sulfate.—The report on this topic has been published (*This Journal*, **29**, 280, 1946). The Associate Referee recommends that Methods I and II as published, with slight modifications, be adopted as tentative. He recommends further that the subject be closed.

The Referee concurs in both recommendations.

Phenolphthalein in Presence of Bile Salts.—One report has been published (*This Journal*, **27**, 353, 1944). Further work involving collaborative tests was carried out this biennium. The recoveries averaged 96.6 per cent. The Associate Referee believes that this is about as close as may be expected for this difficult separation. He recommends that the method used by himself and his collaborators be adopted as tentative and that the subject be closed.

The Referee concurs.

Quinacrine Hydrochloride (*Atabrine*).—The Pharmacopoeia describes this salt and its tablets and provides assays for both. The Associate Referee and his collaborators have employed a shorter method than the U.S.P. procedure. The results are good. The Associate Referee recommends that the short method be adopted as an alternative tentative method.

The Referee concurs.

Demerol (*Isonipecaine*).—One report has been published (*This Journal*, **28**, 711, 1945). No work was reported during the biennium.

It is recommended that the subject be continued.

Sulfanilamide and Related Products.—During the last six or seven years a host of synthetic substances more or less related to sulfanilamide has been introduced to medicine. Methods for the identification and determination of some of them have been adopted by the Association and several have been admitted to the *Pharmacopoeia* or the *National Formulary*. Of late years penicillin and streptomycine have replaced the "sulfa drugs" to some extent. Three years ago an Associate Referee was appointed at his own request to study the "sulfa" drugs as a group, but no report has been received. Since the most important of the "sulfa" drugs have been admitted to the *Pharmacopoeia* or *National Formulary* and methods provided for their identification and assay, it would appear superfluous to extend the list in the *Book of Methods*.

Therefore, it is recommended that the subject be dropped.

Propadrine Hydrochloride.—This topic was assigned three years ago but no report has been received. It is recommended that the subject be continued.

Carbromal.—No report was submitted except a verbal statement that no work had been carried out. The Associate Referee is no longer a member of the Association.

The Referee recommends that the subject be continued.

Phenothiazine.—Two reports have been published[1,2] and an electrophotometric method adopted. The Associate Referee requested that the subject be continued in order to secure more data. Later he resigned without submitting a further report and another Associate Referee was appointed. No report has been submitted by the present Associate Referee.

It is recommended that the subject be closed.

Dihydrocodeineone Hydrochloride.—This synthetic alkaloidal salt was assigned for study two years ago. No formal report has been received. The Associate Referee reported verbally that the substance behaved analytically like codeine salts but that no method of assay had been drawn up for collaborative trials. The Associate Referee is changing his employment and will probably be no longer in Washington. He will remain a member of the Association.

The Referee recommends that the subject be continued.

Methylene Blue.—This topic has been under consideration for some years. The *Pharmacopoeia* has a method for the assay of the compound by precipitation as the perchlorate. The Associate Referee attempted to apply the perchlorate method to mixtures but the results have not been entirely satisfactory.

Since methylene blue has been replaced in medicine, to a considerable extent, by the sulfa drugs and penicillin, the Referee questions the advisability of continuing the subject.

Effervescent Sodium Phosphate.—Directions for the manufacture of this preparation are given by the *U. S. P.* XII, but no method of assay is provided for the finished product. Since effervescent salts are subject to many fortuitous circumstances during manufacture and storage, the necessity for an assay is obvious.

It is recommended that effervescent sodium phosphate be studied.

Hydrastine Hydrochloride.—This salt is described in N.F. VII but no assay is provided. It is recommended that hydrastine hydrochloride be studied.

Phenylmercuric Acetate.—This compound has come into extensive use as a germicide during the past few years. It is frequently exhibited in the form of capsules or in jellies. The Association has not adopted any methods for the assay of this preparation.

It is recommended that phenylmercuric acetate be studied.

Potassium Mercuric Iodide.—This salt is marketed in the form of tablets or discs. It is not described in the U.S.P. or in the N.F. It is described in N.N.R. and methods of assay for iodine and mercury are provided.

It is recommended that potassium mercuric iodide tablets be studied.

Sparteine Sulfate.—This salt has been included in N.F. VII but no

[1] *This Journal*, **27**, 343 (1944).
[2] *Ibid.*, **28**, 696 (1945).

assay is provided for it. It is recommended that sparteine sulfate be studied.

Trichloroethylene.—Trichloroethylene is described in the *U. S. Pharmacopoeia* XII. A rubric for purity is required, but no assay is prescribed. The Association has adopted no method. It is recommended that trichloroethylene be studied.

No report was given on phenothiazine.

REPORT ON PLASMOCHIN

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

The work on this subject was continued this year with a collaborative study of plasmochin tablets. The proposed method depends essentially on a determination of the plasmochin base by titration with standard acid. This procedure differs from the method described in the U.S. Pharmacopoeia under *Pamaquine Naphthoate*, in which case the titration is made with sodium nitrite solution. It is noted that the British *Pharmacopoeia* prescribes a limit for 6-methoxy-8-aminoquinoline in Pamaquine Naphthoate, whereas there is no corresponding test in the U.S.P. It has been reported by Ballard and Pierce,[1] that if this impurity were present it would not influence the acid titration, but would yield a higher apparent base content by the U.S.P. method.

The collaborative sample was prepared using Plasmochin Naphthoate and inert tablet mixture obtained through the cooperation of the manufacturer. Analysis of the Plasmochin Naphoate by acid titration, nitrite assay, and methoxyl determination showed the presence of 43.5 per cent plasmochin base. The collaborative sample, a 20 per cent mixture, therefore contained a theoretical content of 8.70 per cent of the base. A test for 6-methoxy-8-aminoquinoline was negative. Results are given in the table below.

The details of the method are given under "Changes in Methods of Analysis," *This Journal*, **30**, 86 (1947).

COMMENTS OF COLLABORATORS

C. F. Bruening.—No difficulty was encountered except that the end point was difficult to ascertain. It was difficult to detect the first definite purple tint in the presence of the orange color of the solution. Apparently the detection of the end point may be made with rapidity and accuracy after considerable experience has been obtained with the method, but the end point is generally inferior to the usual

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[1] *Quart. J. Pharm. & Pharmacol.*, **XVII**, 30 (1944).

RESULTS AND COMMENTS

Collaborator	*Plasmochin Base Found*	*Recovery*
	per cent	*per cent*
C. F. Bruening,	8.82	101.4
Baltimore	8.00	101.1
A. L. Diamond,	8.70	100.0
New York	8.70	100.0
A. Kramer,	8.70	100.0
New York	8.74	100.4
D. J. Miller,	8.87	102.0
Buffalo	8.78	100.9
C. S. Purcell,	8.00	92.0
Boston	8.00	92.0
L. H. Welsh,	8.66	99.5
Washington, D. C.	8.73	100.3
Associate Referee	8.77	100.8
	8.74	100.4

end points obtained on alkaloids such as codeine, morphine, etc., when using methyl red and other indicators.

D. J. Miller.—Final volume of aqueous solution 100–105 ml. Extraction made with four 30 ml portions of chloroform. A fifth 30 ml extract was negative. Some difficulty was encountered in completely wetting the powder. During washing there was noted some particles which appeared to be more yellow in color than the precipitate. A few drops of 10% HCl was added to the undecanted precipitate to decompose any plasmochin napthoate possibly present.

C. S. Purcell.—9 extractions of 15 ml were found necessary. End point of titration lacks precision. Is a reddish-brown rather than a purple. No other difficulty experienced with the method.

L. H. Welsh.—Although values are expressed to the second decimal place, the end point was so poor the results are considered to be only approximations. Selas crucibles were used in filtering the acid extracts. The filtration proceeded slowly. Removal of the 5 ml of chloroform after addition of standard acid was a slow process. Unless there is danger of loss of plasmochin because of volatility, it would be advantageous to evaporate to a much smaller volume before adding the acid.

DISCUSSION OF RESULTS

The results on the sample were generally satisfactory, although in one case there was a discrepancy greater than usually expected in a method. An additional precaution has been added to the final draft of the method to avoid prolonged heating of the acid solution. This may have caused some of the difficulty with the end point referred to in the collaborators comments.

RECOMMENDATIONS*

It is recommended—

(1) That the method of assay for plasmochin naphthoate tablets be adopted as a tentative method.

(2) That the study be discontinued.

No report was given on 8-hydroxyquinoline sulfate, or on methylene blue.

REPORT ON METRAZOLE

By L. E. WARREN* (Drug Division, Chemical Section, Food and Drug Administration, Washington, D.C.), *Associate Referee*

Metrazole has been under consideration for several years. Two reports have been published[1,2] one recently, but no methods have been adopted by the Association. Although considerable experimental work had been done, it was decided to continue the subject since no meeting was held in 1945. This year two additional collaborators were obtained. One of the collaborators of last year did not participate. Some, but not all, new samples were prepared.

The collaborators were directed to assay the metrazole solutions by Method I[2] and the tablets by Method III, as already published[2] after corrections and improvements had been made.

Two collaborators assayed a solution of known content. The findings ranged from 89.2 to 101.8 per cent of theory. The results by a third collaborator were somewhat larger. In like manner a prepared tablet mixture assayed from 92.9 to 102.9 per cent of its known content. The findings are recorded in Table 1.

Some difficulty was encountered in obtaining a crystalline residue satisfactory for weighing. Repeated alternate evaporations with anhydrous ether and drying over sulfuric acid usually solved the problem.

The results in general were reasonably good.

In order to ascertain whether serious decomposition takes place during the analytical procedure an artificial tablet mixture was made up from lactose, starch, talc, and metrazole recovered from analyses. This mixture was analyzed by Method III in the usual way. The metrazole recovered was dried at 40°. The results indicated that the recoveries were 100.6, 102.0, and 101.3 per cent.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 30, 46 (1947).
* Present address: 2 East Raymond St., Chevy Chase, Md.
[1] *This Journal*, 25, 790 (1942).
[2] *Ibid.*, 29, 296 (1946).

TABLE 1.—*Collaborative results*

METHOD	PREPARATION	COLLABORATOR B		COLLABORATOR C		COLLABORATOR D	
		FOUND	RECOVERY	FOUND	RECOVERY	FOUND	RECOVERY
			per cent		*per cent*		*per cent*
I	Solution A					5.59	89.2
	6.265 Gm/100 ml					6.05	96.6
						5.94	94.8
I	Solution A-1	9.18	99.2	9.32	100.7		
	9.2508 Gm/100 ml	9.23	99.8	9.32	100.7		
I	Solution B	10.07	100.7	10.18	101.8	9.58	95.8
	10.00 Gm/100 ml	10.07	100.7	10.04	100.4	9.41	94.1
						9.15	91.5
						9.78	97.8
I	Solution C	10.00	100.0	10.12	101.2	9.27	92.7
	10.00 Gm/100 ml	10.02	100.2	10.08	100.8	9.55	95.5
III	Tablet Mixture	24.35	97.9			23.52	94.6
	D—24.87 Gm/100 gm	24.46	98.4			24.02	96.6
						24.17	97.2
III	Commercial Tablet					24.91	96.5
	ca 25.79 Gm/100 gm					22.95	92.9
						24.14	93.6
						24.26	94.1
III	Tablet Mixture			25.04	100.6		
	D^1—24.87 Gm/100 gm			25.32	101.8		
				25.20	101.3		
				25.61	102.9		

No report was given on sulfanilamide derivatives.

REPORT ON PHENOLPHTHALEIN IN PRESENCE OF BILE SALTS

BY RUPERT HYATT (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Two years ago the Associate Referee submitted a report on the determination of phenolphthalein in the presence of bile salts (*This Journal*, **27**, 353). At that time no collaborative work had been done. An extraction and iodine precipitation method used by the Associate Referee apparently gave good results. However, further work was seen to be necessary since it was difficult to extract the phenophthalein from some types of products.

It was found preferable to treat the powdered material with alkali to prevent occlusion. The filtration was omitted because of the difficulty of removing all the phenophthalein from the paper.

Several collaborative samples were sent out and the reports received. One collaborator suggested the use of ether for the extraction. This was found to be suitable but did not yield better results than the chloroform-ether mixture. This method was revised in some particulars and is published in *This Journal*, **30**, 89 (1947).

The material used was a mixture somewhat typical of commercial preparations. It contained phenolphthalein, sodium glycocholate, sodium taurocholate, ext. cascara sagrada, aloin, starch and lactose. The theoretical phenolphthalein content based on composition and assay was 11.08 per cent.

Results obtained by the method are listed in this table:

Collaborator	*Per Cent Phenolphthalein*	*Per Cent Recovery*
S. D. Fine, Cincinnati	10.59	95.6
	10.57	95.4
Daniel Banes, Chicago	10.57	95.4
	10.58	95.5
	10.83	97.7
Mary A. McEniry, St. Louis	11.0	99.3
	10.7	96.6
George McClellan, New Orleans	10.59	95.6
	10.24	92.4
	10.43	94.1
R. Hyatt, Cincinnati	11.14	100.5
	11.20	101.1
Average	10.70	96.6

Though the results are not in perfect agreement the method seems to be useful and it is believed that good results will be obtained on the average sample.

It is recommended* that the procedure be adopted as a tentative method and the topic closed.

No reports were given on the following subjects: atabrine (chinacrin, quinacrine), demerol, propadrine hydrochloride, carbromal, dihydrocodeinone, butacaine sulfate, spectrophotometric methods.

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* For the report of Subcommittee B and action by the Association, see *This Journal*, 30, 46 (1947).

REPORT ON MISCELLANEOUS DRUGS

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

For the past year ten topics were assigned to the Referee. Each topic and recommendation will be discussed separately.

Organic Iodides.—No report was received from the Associate Referee. The Referee recommends that the subject be continued.

Compound Ointment of Benzoic Acid.—The Associate Referee reports that because of extended illness collaborative samples were not submitted for study, but he recommends that the subject be continued. The Referee concurs.

Glycols and Related Compounds.—The Associate Referee reports that he is confident that he can bring this work to a quick conclusion as soon as the pressure of official work lessens. The Referee recommends that this subject be continued.

Alkali Metals in Drugs.—A report was submitted by the Associate Referee on work so far done on this subject. The Associate Referee also requested that he be relieved of this position and the work be assigned to someone else. The Referee recommends that this topic be reassigned and that the report submitted by the Associate Referee be made available to the new appointee.

Preservatives and Bacteriostatic Agents in Ampul Solutions.—A report was received. The Referee recommends that this subjected be continued.

Separation of Bromides, Chlorides, and Iodides.—No report was received. The Referee recommends that this subject be continued.

Phosphorus, Calcium, and Iron in Vitamin Preparations.—The Associate Referee reports that there are available in the A.O.A.C. *Book of Methods*, methods for the determination in macro and micro amounts of phosphorus, calcium, and iron, which would be applicable for the determination of phosphorus, calcium, and iron in vitamin tablets. The Associate Referee recommends that the subject be discontinued. The Referee recommends that the subject be continued and a further study made of the applicability of these methods.

Mercury Compounds (Ethanolamine Methods).—No report was received. The Referee recommends that the subject be continued.

Microchemical Tests for Alkaloids and Synthetics.—No report was received. The Referee recommends that the subject be continued.

Effervescent Antipyrine with Caffeine.—The Associate Referee recommends that this subject be discontinued for the following reasons:

(1) The A.O.A.C. has a method for antipyrine and caffeine which would, no doubt, be satisfactory for this mixture.

(2) No information could be obtained, from a search of the literature, that this mixture is prepared for use in this country.

The Referee concurs that this subject be dropped.

The Referee recommends* that the topic "Separation and Determination of Certain Drugs by Superheated Steam" be studied.

No reports were made on the following subjects: microchemical tests for alkaloids and synthetics, mercury compounds (ethanolamine methods), separation of bromides, chlorides, and iodides, organic iodides, compound ointment of benzoic acid.

REPORT ON ALKALI METALS IN DRUGS

By W. C. Woodfin (Food and Drug Administration, Federal Security Agency, Pittsburgh, Pa.), *Associate Referee*

This topic was assigned to the present Associate Referee in the Spring of 1941 as a result of a small amount of experimental work done on the determination of lithium in a drug consisting of a mixture of a small amount of lithium benzoate and large amounts of sodium phosphate, sodium thiosulfate, sodium benzoate, and sodium chloride. The method used was that of the A.O.A.C. with a final weighing of the lithium as sulfate, but complete satisfaction was not felt with this method because of the possibility of loss of some of the lithium sulfate by decrepitation during the firing of the lithium sulfate.

A search of the literature was made and two methods for lithium were selected as being those most likely to yield a satisfactory method for small amounts of lithium. No work has yet been done on methods for sodium and potassium as there appeared to be satisfactory methods for these two elements in the A.O.A.C. *Book of Methods* (1) which were included into the drug section by the Associate Referee on bromide preparations (2). As a result of these considerations this report will be limited to the work which was done on the determination of lithium.

The two methods selected for further study were that of O. Procke and A. Slouf (3) based on the precipitation of lithium potassium periodate with subsequent solution of the precipitate and titration of iodine released from KI by the iodate radical; and the method of Yu.A. Chernikhov, T. A. Uspenskaya, and R. S. Anan'ina (4) based on the precipitation of the lithium as lithium–zinc-uranyl-acetate from a strong acetic acid medium with subsequent weighing of the dried salt. Some experimental work indicated that the latter method gave more consistent and concordant results in this laboratory so the remainder of the work was concentrated on this method.

The method used is as follows:

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* For report of Subcommittee B and action by the Association, see *This Journal*, 30, 46 (1947).

REAGENTS

Precipitating Reagent.—Dissolve 30 grams of zinc acetate in 250 ml of 60% acetic acid with heating to 35–40°C., adding 30–35 g of uranyl acetate, adding some lithium-zinc-uranyl-acetate, letting the solution stand overnight, and filtering before use.

Wash Solution.—Saturate 95% alcohol with some of the lithium-zinc-uranyl-acetate, filtering just before use.

PROCEDURE

After separating the lithium from the other alkali metals as directed in **XXXVII, 66** (*Methods of Analysis, 1940*, p. 538) evaporate the alcohol-ether solution of the lithium chloride to a small volume and add ca 5 ml of 1–2.5 HCl and continue the evaporation to dryness. Take the lithium chloride up in water and dilute to 100 ml. Transfer an aliquot containing 1– 4 mgm of lithium to a 50 ml beaker, evaporate to dryness, take up the residue in 1 ml water, add 25 ml of the precipitating reagent, stir vigorously ca 2 minutes, cover with watch-glass, and allow to stand at least 30 minutes.

Prepare a Gooch or sintered glass crucible of fine porosity as follows: Wash with 5 portions of 2 ml each of the alcohol wash solution, and then with two 5 ml portions absolute ether, sucking dry between each portion of wash liquid, dry ca 10 minutes at 100°C., cool in an efficient desiccator and weigh.

Filter off the precipitate in the prepared crucible, using some of the mother liquor to complete the transfer if necessary. Complete the transfer of the precipitate and wash the precipitate in the crucible with 5 portions of the alcohol wash solution, using ca 2 ml each time, wash the precipitate in the crucible with two 5 ml portions of anhydrous ether. Dry in oven at 100°C. ca 10 minutes, cool in desiccator, and weigh. Weight of precipitate $\times 0.00456$ gives the weight of lithium in the aliquot used.

Recovery experiments using 1, 2.5, and 5 mgm of lithium as lithium chloride prepared from reprecipitated lithium carbonate yielded recoveries varying from 97.5 to 99 per cent of the put in amount.

Four determinations on solutions containing from 1.5 to 2.5 mgm of lithium, the lithium content of which was unknown to the analyst, yielded recoveries of 97.5 to 99.8 per cent of the put in amount. Seven solutions containing from 1 to 4 mgm of lithium as lithium chloride, together with the reagents and the directions, were submitted to three of the analysts in the Atlanta Station and they reported recoveries ranging from 97.9 per cent to 103.9 per cent of the put in amounts of lithium.

I wish to acknowledge, with thanks, the cooperation of Dr. W. H. Hall, Editor of the Analytical Section of Chemical Abstracts, for furnishing a translation of the method of O. Procke and A. Slouf; and to Mr. Messrs. A. M. Henry, C. D. Schiffman, R. E. O'Neill, and N. E. Freeman, of the Atlanta Station of the Food and Drug Administration, for their advice and assistance in this work.

REFERENCES

(1) *Methods of Analysis*, 1940, p. 129, or p. 438. Sodium and Potassium—Official.
(2) *This Journal*, **XXV**, p. 847 (1942).
(3) O. Procke and A. Slouf. "Estimation of Lithium as Lithium Potassium Ferric Periodate." Collection *Czech Chem. Commun.*, **11**, 273–94 (1939).

(4) YU. A. CHERNIKHOV, T. A. USPENSKAYA, and R. S. ANAN'INA. Zavodskaya Lab. 9, No. 1, 28–33 (1940): "Determination of Lithium in Light Alloys," as abstracted in *Chemical Abstracts* 35, No. 22, p. 7872 (1941).

REPORT ON GLYCOLS AND RELATED PRODUCTS

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

Numerous investigations have been made on the qualitative and quantitative estimation of the pure glycols. (1)–(8).

It was decided that this year the ground work for this subject should be of primary concern; and that this should consist of developing general methods for separating the glycols from mixtures of which they are a part.

The glycols are used in food and medicinal products as solvents, moistening agents, and preservatives. During the war years Propylene Glycol was officially used as a replacement for glycerin in a number of medicinal products. It is the only glycol permitted in the manufacture of food and medicinal products. However, to detect the inclusion of other glycols, it was decided to devise means for separating the glycols from these products and for identifying them.

The work was concerned primarily with Propylene, Ethylene, and Diethylene Glycols, 'Carbitol' (9) and Glycerin. All are soluble in water and ethyl alcohol and slightly soluble in the hydrocarbon solvents with the exception of carbitol, which is soluble in ethyl alcohol and the hydrocarbon solvents. Wilson (10) and others in the U. S. Food and Drug Administration have used immiscible solvent extractions for the separation of glycols and glycol ethers from certain mixtures. No one general method can be set up for all food and medicinal products because of the varying nature of their composition; but for each class of product, such as medicinal elixirs, flavoring extracts, tooth pastes, cosmetic creams, and a long list of others, a general method can in most instances be adapted to the product. Shupe (6) has worked on the separation of carbitol in mixtures.

For the experimental work Propylene, Ethylene, and Diethylene Glycols were distilled in the laboratory and a constant boiling fraction removed. Physical constants were determined as follows;

	Sp. Gr. 25/25°C.	*R.I. 25°C.*	*B.P. °C.*
Propylene Glycol	1.0359	1.4313	186.6–187.6
Ethylene Glycol	1.1136	1.4299	197.2–198
Diethylene Glycol	1.1167	1.4450	243.6–246.6

All the distillates were odorless and colorless. Acetylation values were not determined at this time.

Glycerin and Propylene Glycol were first used in experimental mixtures, since they are the ones most likely to be found in food and medicinal

products. Propylene Glycol is miscible with chloroform, whereas glycerin is but slightly soluble in chloroform.

Experiment No.	*Mixture*		*Propylene Glycol Recovered*	
1	Propylene Glycol	25 ml	20 ml.	80%
	Glycerin	25 ml		
	Water	50 ml		
2	Phenobarbital	0.4 gm.		
	Tr-Sweet Orange Peel	3.0 ml		
	Sol. Amaranth	1.0 ml		
	Alcohol	12.5 ml		
	Glycerin	22.5 ml	21+ ml	84%
	Propylene Glycol	25.0 ml		
	Syrup	15.0 ml		
	Water to make	100.0 ml		

In Experiment 1, the mixture was transferred to a Palkin continuous extractor. After 8 hours of extraction with chloroform, the chloroform extract was removed and the chloroform evaporated. The residual material was distilled in a small Ladenburg flask using an air condenser. The distillate had a boiling point of 186.8–187.4°C and an index of refraction of 1.4322 at 25°C.

In Experiment 2, the mixture was extracted in a separatory funnel with petroleum ether to remove essential oils and other materials soluble in this solvent. The mixture was then transferred to the Palkin continuous extractor and extracted with chloroform for 8 hours. The extracted material was distilled in a small Ladenburg flask, as above, after evaporating the chloroform. Recovery was practically the same as from the aqueous mixture, and physical constants were: boiling point 187°C, index of refraction, 1.4320 at 25°C.

Separation of the glycol by direct distillation was found to be impossible because of charring of the sugar in the preparation. Other methods are at present under consideration but no satisfactory method has as yet been developed.

It is recommended* that the subject be continued.

REFERENCES

(1) *Bull. Soc. Chim.*, (4) **43**, 683 (1928).
(2) *Quart. J. Phar. and Pharmacology*, **8**, 390 (1935).
(3) *Ind. Eng. Chem., Anal. Ed.*, **9**, 509 (1937).
(4) *Analyst*, **63**, 259 (1938).
(5) *Ind. Eng. Chem., Anal. Ed.*, **12**, 384 (1940).
(6) *This Journal*, **24**, 936 (1941).
(7) *Ibid.* **26**, 99 (1943).
(8) *Ibid.*, **26**, 250 (1943).
(9) Trade mark name, Carbide and Carbon Chemicals Corp., New York, N. Y.
(10) Private communication.

* For Report of Subcommittee B and action by the Association, see *This Journal* **30**, 47 (1947).

REPORT ON PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS

By Charles N. Jones (U. S. Food and Drug Administration, Federal Security Agency, New York, N. Y.),
Associate Referee

The following compilation of preservatives and bacteriostatic agents in ampul solutions has been compiled from the *U. S. Pharmacopoeia XII*, the *National Formulary VI*, and the *New and Nonofficial Remedies* of 1944. It is not intended that this list be considered as a complete list of all such agents, nor as a recommendation for any particular one. The grouping into the few main categories is to aid in the examination of the chemical characteristics of the entire list of agents.

Group	*Agent*	*Minimum Concentration*	*Maximum Concentration*
A. Phenolic	Phenol	0.2%	0.5%
	Cresol	0.15%	0.4%
	n-butyl parahydroxybenzoate	0.01%	
B. Neutral	Chlorobutanol	0.3%	0.5%
	Benzyl alcohol	2.0%	5.0%
	Camphor	2.0%	
	Glycerin		
	Thiourea		
C. Mercurial	Merthiolate	1:10,000	1:1,000
	Metaphen	1:20,000	
	Phenyl mercuric borate	1:100,000	1:15,000
D. Inorganic	Sodium bisulfite	0.1%	0.3%
	Sulfurous acid	0.06%	
	Acetone sodium bisulfite	0.4%	

Acetone sodium bisulfite is included in the list of inorganic preservatives since it is the bisulfite group which exhibits the antioxidant properties.

Qualitative tests for the presence of a preservative depend largely upon the application of specific reagents for the suspected preservative. There are few solutions with more than one preservative, and this simplifies the identification, since only general separations may be made. There can be no rigid procedure for the chemical identification of all preservatives, since the nature of the substance requiring the preservative must be taken into consideration. Care must be exercised then in the interpretation of any result where there may be interfering substances present. Bearing in mind these warnings, the following outline is submitted as a guide for the identification of most of the preservatives in the above list.

Extract about 5 ml of the ampul solution, which has been acidified with dilute sulfuric acid, with three 10 ml portions of ether. The combined ether extracts (I) are washed with 5 ml of water, adding the wash water to the main aqueous solution (II).

Approximately one-third of the ether extract (I) is evaporated to near dryness in a current of warm air, and the remaining ether is allowed to evaporate without heat. The odor of the residue may be that of phenol, cresol, or benzyl alcohol. Take this residue up in a few drops of alcohol, dilute to about 5 ml with water, and divide into two portions. Underlay the first portion with 3 ml of concentrated H_2SO_4, to which has been added 2 drops of formaldehyde solution (Marquis reagent). Formation of a red ring indicates phenol or cresol. Parahydroxybenzoic acid and its esters do not give this test, while many phenols do. To the second portion add 2.5 ml of Millon (1) reagent, and heat for half an hour in boiling water. The formation of a red color shows presence of phenol, or the esters of parahydroxybenzoic acid. Many substances interfere with this test, such as proteins, aniline, and compounds which give phenol or aniline on boiling with nitrous acid (2). Pure cresols do not give the color, but commercial cresols, contaminated with phenol, do.

Chlorobutanol is found by taking a second portion of the ether extract (I) and evaporating to near dryness. Add one drop of aniline, 5 ml of NaOH T.S., and heat to boiling. The odor of phenylisocyanide indicates chlorobutanol (3).

For benzyl alcohol, the third portion of the ether extract is evaporated and about 5 ml of KM_nO_4, T.S., acidified with dil. H_2SO_4, is added. The characteristic odor of benzaldehyde shows the presence of benzyl alcohol (4).

The aqueous phase II may contain thiourea, the inorganics, and the mercurials. To test for thiourea, one ml of the aqueous solution II is diluted to about 50 ml, and 5 ml is added to 5 ml of a solution of 3 grams sodium azide in 100 ml of 0. 1 *N* iodine solution. Evolution of nitrogen is indicative of a thioketone or mercaptan (5). The presence of thiourea can be confirmed by the Deniges test (6), which consists of boiling 5 ml of the diluted solution with 2 ml of NaOH T.S., followed by 2 drops of a solution of sodium nitroprusside. Thiourea gives a purple color.

Glycerol may be detected, as suggested by Levine (7), by oxidizing it with bromine water, the excess bromine boiled off, and cooled. Add 2 ml of H_2SO_4, followed by 0.1 ml of a 5% solution of resorcinol, or other suitable phenol. If resorcinol is used, a wine red color indicates glycerine.

The inorganics, all sulfite compounds, are readily detected by the bleaching of a drop of acidified permanganate solution suspended over a warmed acid portion of the ampul solution.

Distinctive qualitative tests have not been developed as yet on the mercurial class of preservatives.

Camphor is found only in oil solutions, and its odor is characteristic enough for identification.

The above outline has been tested only on control solutions, where interfering substances are absent. It has not been tested collaboratively, nor have there been any quantitative applications.

It is recommended* by the Associate Referee that the above qualitative outline be subjected to further study, that qualitative tests be developed for the mercurial type of preservative, and quantitative methods for all preservatives be developed.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), **7.1 (b)** p. 93.
(2) FEIGL, F., Spot Tests. 276 (1939) (Nordemann).

* For report of Subcommittee B and action by the Association, see *This Journal*, **30**, 47 (1947).

(3) *U. S. Pharmacopoeia*, **XII**, 129 (1942) (Mack).
(4) *New and Non-Official Remedies*, **77** (1944).
(5) FEIGL, F., Spot Tests, 291 (1939).
(6) *Merck's Index*, 5th Ed., Test 903, p. 691.
(7) *J. Am. Pharm. Assoc.*, **XX**, 540 (1931).

No reports were made on phosphorus, calcium, and iron in vitamin preparations, or on effervescent antipyrine with caffeine.

No general report was made on the subject of drug bioassays, including enteric coatings, posterior pituitary, ergometrine (ergonovine), or digitalis preparations.

No general report was made for microbiological methods, nor on specific subjects of canned fishery products, canned meats, canned vegetables, canned tomatoes and other acid vegetables and fruit products, sugar, eggs and egg products, nuts and nut products, frozen fruits and vegetables.

No report was made on microchemical methods.

TUESDAY—AFTERNOON SESSION

REPORT ON OILS, FATS, AND WAXES

By J. Fitelson (U. S. Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

No reports on methods for stability of fats and on peanut oil have been submitted by the Associate Referees. The tentative method for squalene and the S.P.A. method for unsaponifiable matter were again studied collaboratively and the findings warrant adoption of these methods as official.

RECOMMENDATIONS*

It is recommended—

(1) That the tentative S.P.A. method **31.40** (*Methods of Analysis*, 1945, p. 504) be adopted as an official method (first action).

(2) That the chromatographic procedure for the purification of the unsaponifiable matter be studied.

(3) That the official F.A.C. method for unsaponifiable matter (*Methods of Analysis, A.O.A.C.*, 1945, p. 504 (sections **31.37, 31.38,** and **31.39**) be deleted (first action).

(4) That the tentative method for squalene **31.41–31.43** (*Methods of Analysis, A.O.A.C.*, 1945, p. 505) be adopted as official (first action) and that this subject be closed.

(5) That studies on methods for the determination of the stability of fats be continued.

(6) That studies on methods for the estimation of peanut oil be continued.

REPORT ON UNSAPONIFIABLE MATTER

By G. Kirsten (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

The results of the second collaborative study of the present official method and the tentative S.P.A. method for the determination of unsaponifiable matter in fats have been previously reported (*This Journal*, **29**, 248 (1946). These results confirm the conclusion reached after the first study, that the S.P.A. method is superior to the present official method.

It is therefore recommended†—

(1) That the tentative S.P.A. method **31.40** (*Methods of Analysis, A.O.A.C.*, 1945, p. 504) be made official (first action).

(2) That the present official (F.A.C.) method for unsaponifiable matter,

* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 52 (1947).
† For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 52 (1947).

31.37–31.39, incl. (*Methods of Analysis, A.O.A.C.*, 1945, p. 504) be deleted (first action).

(3) That the chromatographic technique of Sylvester *et al* (*Analyst*, **70**, 295 (1945) be studied with view of its adoption as an alternative to the washing procedure for the purification of the unsaponifiable extract.

No report was made on peanut oil.

REPORT ON OLIVE OIL

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

The results of the 1945 collaborative studies on the method for squalene (*Methods of Analysis, A.O.A.C.*, 1945, p. 505) have been previously reported, *This Journal*, **29**, 247 (1946). The findings confirmed the satisfactory results obtained in the first collaborative work (*This Journal*, **28**, 283).

It is therefore recommended*—

(1) That the tentative method for squalene **31.41–31.43**, be adopted as official (first action), and

(2) That further work on this subject be discontinued.

No report was made on stability of fats or on antioxidants.

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

By Margarethe S. Oakley (Maryland State Department of Health, Baltimore, Md.), *Referee*

The field of preservatives and artificial sweeteners for foods has made great strides within the past decade, but the chapter on preservatives and artificial sweeteners in the A.O.A.C. *Book of Methods* has remained almost static. There are several reasons for this unsatisfactory condition.

The chapter now consists of qualitative and quantitative tests for salicylic acid, benzoic acid, saccharin, sulfurous acid, boric acid, and borates. It includes, also, a plethora of qualitative tests for formaldehyde without the most sensitive and modern one, and qualitative tests for fluorides, beta-naphthol, abrastol, and dulcin. It ends with a quantitative method for formic acid which could probably be advantageously superseded by the Hillig method for this compound.

* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 52 (1947).

The Referee feels that the chapter should contain, in addition to the older tests for preservatives, methods for the determination and/or detection of monochloracetic acid, dichloracetic acid, thiourea, esters of p-hydroxybenzoic acid, propionates, diacetates, and quaternary ammonium compounds, The study of antioxidants now being used in fats has been assigned to the section on oil, fats, and waxes.

It is to be hoped that an Associate Referee for formaldehyde will be found this year, who will sort out the methods and bring this part of the chapter up to date.

A method for monochloracetic acid has been developed by John B. Wilson of the Food and Drug Administration, but study had not been completed for its adoption in the sixth edition of the *Methods of Analysis*.

Two papers on the determination of the quaternary ammonium compounds in foods appeared in the August (1946) issue of the A.O.A.C. *Journal*, one by T. H. Harris and the other by John B. Wilson. Both of these men are Food and Drug Administration chemists and it would be very desirable if one of them could be persuaded to continue these studies through the collaborative stage so that a method could be made available to the *Book of Methods*.

During the past year E. B. Boyce resigned as Associate Referee on benzoates and esters of benzoic acid and W. J. McCarthy, U. S. Food and Drug Administration, Cincinnati, was appointed in his place. He has submitted a preliminary summary of his field and expects to cover p-hydroxybenzoic acid and the vanillic acid ester. Dr. David W. Horn resigned as Associate Referee on formaldehyde, but up to this time no one has been appointed to cover this topic. C. E. Hynds resigned as Associate Referee on sulfites, and it is recommended that this subject be dropped pending further developments in this field.

Lack of time to devote to these problems was the reason given for the resignation of each of these three Associate Referees. It is also for this reason that the chapter on Preservatives and Artificial Sweeteners has had such difficulty in endeavoring to catch up with what should already be in the process of being studied. The problems are awaiting the securing of Associate Referees to handle them.

The field of artificial sweeteners has been supplemented recently by preparations designed to split sucrose to invert sugar, which furnishes more sweetness; however, in the knowledge of the Referee no new sweetener has appeared.

RECOMMENDATIONS*

The Referee recommends—

(1) That work be continued on benzoates and esters of benzoic acid.

(2) That saccharin in baked goods be studied.

* For report of Subcommittee C and action by the Association, see *This Journal*, 30, 52 (1947).

(3) That work be initiated for the detection and/or determination of antioxidants in fats.

(4) That studies be undertaken on methods for the detection and/or determination of quaternary ammonium compounds, mold inhibitors—propionates, diacetates, thiourea, and dichloracetic acid.

(5) That the subject of sulfites in foods be dropped for the present.

(6) That following **32.30** in the chapter, which is the qualitative test for fluorides, the words "quantitative, see **29.22** through **29.33**" be inserted.

(7) That a referee be appointed to continue the work on qualitative tests for formaldehyde.

(8) That the monochloracetic acid method be submitted to collaborative study.

REPORT ON BENZOATES AND ESTERS OF BENZOIC ACID

By W. J. McCarthy (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati 2, Ohio), *Associate Referee*

Since the last A.O.A.C. meeting, there appeared in the Food Industries Journal, December, 1945, an article on vanillic acid esters as food preservatives, by Pearl and McCoy of the Institute of Paper Chemistry, Appleton, Wisconsin. This article points out the value of the esters as a preservative, and in view of the evident enthusiasm of the authors we might expect the use of vanillic acid ester (ethyl vanillate) in food products in the near future. Accordingly, a method for detection and possibly quantitative determination of the ester in a foodstuff is desired. In *The Analyst*, February, 1946, there appears a colorimetric method for the determination of para hydroxybenzoic acid and its esters. This method appears to have possibilities. A local Cincinnati commercial laboratory has done a small amount of work on a colorimetric method for the estimation of ethyl vanillate in food and advances the thought that its presence can be claimed as a flavor if a situation arises where its presence is determined positively. Owing to laboratory circumstances, work on this subject has been confined to perusal of the literature.

REPORT ON SACCHARIN

By Margarethe S. Oakley (Maryland State Department of Health, Baltimore, Md.), *Associate Referee*

In 1924 and in subsequent years it was suggested in the recommendations of Committee C that the sublimation process be applied to saccharin. This is a method of separation or purification instead of a chemical de-

termination of one of the constituents of the compound, as the official method prescribed. The advantage of sublimation is, of course, that it extracts the saccharin in a more refined state, but it is still present as saccharin which can be tasted or qualitatively tested by determination of the melting point, or by either of the qualitative tests in the *Book of Methods*.

Preliminary groundwork of 1943 having shown promise, the method was submitted to collaborators in 1944. Directions called for (A) the addition of a quantity of saccharin solution to 200 ml. of a simple soft drink, acidification and extraction with ether, followed by sublimation. To another 200 ml. of the same type of beverage a quantity of a benzoic acid solution was to be added in addition to the saccharin. This sample (B) was extracted three times with 50 ml. portions of carbon tetrachloride with subsequent ether extraction and sublimation. The extraction with carbon tetrachloride was for the purpose of removing the interfering benzoate.

COMMENTS OF COLLABORATORS

A. The sublimates in experiment A (saccharin alone, no carbon tetrachloride extraction) appeared dark-colored and contaminated. In experiment B the sublimates appeared quite pure. It is thought the carbon tetrachloride extraction in B not only removes benzoic acid but also other matter which may interfere, *i.e.*, oleoresin or flavors which are present in the ginger-ale type beverage.

B. On Sample B (saccharin and benzoate solutions) apparently high results were obtained assuming that the benzoate solution did not contribute any saccharin to the mixture. It was noted that crystals, apparently benzoic acid, formed on the top part of condenser of the sublimator (i.e., the crystals had swept past the coldest part of the condenser and collected at the point nearest the connection to the vacuum). Crystal formation at this point indicated considerable volatility of these crystals since with the saccharin of sample A all the sublimate collected at the bottom of the condenser (which is nearest that part of the apparatus where sample was originally placed for sublimation).

The above results indicate to me that benzoic acid was not completely removed by the carbon tetrachloride extraction. Some emulsion difficulty was encountered in extracting, but reasonably clear-cut separations of the solvent were obtained.

However, to remove the benzoic acid still remaining in the residue I heated them at 100°C. for one and two hour periods. At the same time the residues of sample A were heated for the same periods to determine whether saccharin would be lost. The results indicate that no appreciable amount of saccharin is lost when heated at 100°C., while all the benzoic acid is removed. Thus, I suggest this procedure for removing benzoic acid from saccharin in the residues, and it may even be advisable to omit the carbon tetrachloride extraction, although this solvent may be helpful in removing volatile oils, etc.

COMMENT TO THESE SUGGESTIONS

C. It was found by the Associate Referee that neither fractional sublimation, nor extraction with a solvent which did not dissolve saccharin, nor heating of the final residue for an extended period would effectively separate benzoate from saccharin but that a combination of the three would give an acceptable separation; therefore, the method as suggested for tentative adoption is:

METHOD

Acidify 200 ml. of sample with 15 ml HCl. Extract 3 times with 50 ml portions of CCl_4. Discard the CCl_4. Extract the aqueous layer 3 times with 80 ml portions of ether. Allow the ether extract to evaporate to a small volume and transfer to a sublimator with a small amount of ether or alcohol. Evaporate to dryness (at room temperature or on a water bath, depending upon which solvent was used for transferring residue). Sublime residue at a pressure of 1–2 mm. and temperature of 140°C.–160°C. for 1 hour. (Raise temperature slowly so that 140°C. is reached in about ½ hour.) Wash saccharin from the condenser bulb of the sublimator with warm alcohol into a weighed beaker. Repeat the sublimation until no further residue appears on the condensing bulb. Evaporate the alcohol on water bath and heat residue 2 hours at 100°C. Cool and reweigh beaker.

TABLE OF RESULTS OF COLLABORATORS

To be added	0.0450 Gm. Saccharin	0.0450 Gm. Saccharin +0.0450 Gm. Benzoate
Found by		
Analyst A	0.0469 (2 hr. drying at 100°C.)	0.0469 (2 hr. drying at 100°C.)
	0.0467 (2 hr. drying at 100°C.)	0.0472 (2 hr. drying at 100°C.)
Analyst B	0.0497	0.0475
	0.0467	0.0488
Analyst C	0.0532	0.0415
	0.0484	0.0426
Analyst D	0.0431	0.0451
	0.0421	0.0420
Analyst E	0.0481	0.0478
	0.0482	0.0473

Nothing was common to all these determinations except the solutions of saccharin and benzoate used. There were different types of sublimators used by different collaborators, and no two samples of soft drinks were the same; ginger ale, cherry, grape, and cream soda being the flavors used.

These results, although not excellent, are within what might be expected from the other official tests for saccharin and it is recommended that the sublimation method be adopted as tentative and that further work be done on the method in order to widen its scope of application to include more complicated foods, particularly baked goods.

The Associate Referee wishes to express appreciation to the following collaborators for their cooperation.

Charles A. Wood, New York Station, U. S. Food and Drug Administration.
Muriel W. Trusheim, Maryland State Department of Health.
C. H. Bruening, Baltimore Station, U. S. Food and Drug Administration.
A. Zilliox, Chemical Laboratory, State of South Dakota.

No report was given on sulfur dioxide.

REPORT ON MONOCHLOROACETIC ACID IN FOODS

By John B. Wilson, Chemist (U. S. Food Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

It is recommended* that collaborative study of the method for monochloroacetic acid in food be continued.

No report was given for formaldehyde.

REPORT ON STANDARD SOLUTIONS

By H. G. Underwood (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Referee*

New subjects.—Ten standard solutions have been studied to date. These include preliminary studies on bromide-bromate solutions and titanium trichloride solutions—solutions recommended for study in the 1945 Referee's report. It is believed that potassium dichromate solutions are sufficiently important as oxidizing solutions and as primary standards to warrant study by the Association.

Standard sodium thiosulfate solutions.—Although the February 1945 and 1946 issues of *The Journal* list Mr. G. M. Johnson as Associate Referee, the Associate Referee in 1944 and the Referee in 1945 recommended that the subject be closed and the method reported in *This Journal*, **25**, 659, 1942, be adopted as official, first action. There appears to be no reason for re-opening this subject at this time.

Standard buffers.—Dr. Manov is continuing the study of standard buffer solutions and has submitted a progress report. This subject should be continued.

Thiocyanate solutions.—Mr. Deal and the collaborators have obtained concordant results in the standardization of a thiocyanate solution using silver nitrate subjected to a special method of purification. The results were generally in agreement with the theoretical value within one part per thousand. The results warrant adoption of the method as official, first action. Studies by Mr. Deal indicate that solutions of ammonium thiocyanate are less subject to deterioration through mold growth in warm weather than solutions of potassium and sodium thiocyanate.

Potassium permanganate solutions.—Mr. Duggan has reasoned that in actual practice it should be possible to determine a substance by the official A.O.A.C. procedure as accurately as by that recommended by the Bureau of Standards. It will be necessary that comparative results be obtained on a number of different compounds to confirm this view. This subject should be continued.

* For report of Subcommittee C and action by the Association, see *This Journal*, **30**, 53 (1947).

Bromide-bromate solutions.—Mr. VanDame has obtained concordant results in the standardization of a bromide-bromate solution by two procedures. Direct titration against Bureau of Standards arsenious oxide appears to be less subject to error and the more convenient procedure. This subject should be continued.

Titanium tri-chloride solutions.—Preliminary studies of three methods of standardizing a solution of titanium trichloride by Miss Breit indicate that two procedures yield concordant results, while somewhat lower results are obtained by the third. Further study should be made of the standardization procedures and consideration should be given to the stability of titanium trichloride solutions.

RECOMMENDATIONS*

It is recommended—

(1) That the preparation and standardization of potassium dichromate solutions be studied;

(2) That the preparation and standardization of sodium thiosulfate solutions be considered closed;

(3) That the studies on buffer solutions be continued;

(4) That the preparation and standardization of thiocyanate solutions be closed and that the method submitted by Mr. Deal be adopted as official, first action;

(5) That the studies on potassium permanganate solutions be continued;

(6) That the studies on bromide-bromate solutions be continued; and

(7) That the methods for standardization of titanium trichloride solutions be studied further.

No report was given on sodium thiosulfate solutions.

REPORT ON THIOCYANATE SOLUTIONS

By E. C. Deal (U. S. Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Associate Referee*

Work undertaken this year was a continuation of the work begun in 1942 with the hope of finding a suitable method for the direct standardization of thiocyanate solutions. A study of such methods was recommended (1) by the Association in 1941, when it adopted as tentative a method of standardization by reference to a standardized solution of silver nitrate. Experimental work reported (2) in 1942 showed nitrate of silver to be the substance best suited for use as a primary standard in

* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 44 (1947).

determining the strength of thiocyanate solutions. A method of preparation of purified silver nitrate by recrystallizing and fusing was reported at that time. The Associate Referee was able to obtain standardization values agreeing closely with theory. Results on collaborative samples sent out the following year, however, were disappointing.

In 1944 a study was conducted (3) in an effort to find the cause of variations in results reported by different collaborators in 1943. It was suspected that impurities or decomposition products in the silver nitrate were to blame. Certain changes in the method of preparation of the purified silver nitrate were indicated. It was found that a single recrystallization of C. P. grade silver nitrate with subsequent controlled drying and fusing produced a product suitable for use as a primary standard. Titrations using different batches of silver nitrate gave good results in the hands of the Associate Referee. This year, other slight changes were made in the proposed (1942) methods of preparation and standardization. The revised methods are given in detail under "Changes in methods," *This Journal*, **30**, 105 (1947).

COLLABORATIVE STUDY

This year a new solution was prepared from recrystallized, vacuum-dried potassium thiocyanate by dissolving 59.1183 grams of the chemical and making to a volume of 6 liters. The theoretical strength of this solution was 0.10141 normal. Portions of the solution were sent to collaborators with instructions to standardize same according to the revised method. The following table shows the results obtained by the collaborators.

It will be noted that the results generally are in agreement with the theoretical value within one part in one thousand with a tendency towards the higher value, probably because a small amount of the silver was absorbed on the precipitate. The Associate Referee believes the collaborative results show that the method is satisfactory for laboratory use.

A study was made of the keeping qualities of thiocyanate solutions. There is a tendency for mold growth to develop in these solutions in warm weather. It was found that sodium and potassium thoicyanate solutions are more readily affected than solutions of ammonium thiocyanate. Three solutions were prepared in identical manner from sodium thiocyanate, potassium thiocyanate, and ammonium thiocyanate, using portions of the same distilled water for each solution. The solutions were stored at room temperature (approximately 30°C). At the end of two weeks mold growth was well started in the sodium thiocyanate solution, and was beginning to develop in the potassium thiocyanate solution, while the ammonium thiocyanate solution was free from mold growth. Two months later heavy growths of mold were observed in the sodium and potassium thiocyanate solutions but only a trace in the ammonium thiocyanate solution. The titer of the ammonium thiocyanate solution had not changed. It would

Collaborative results—potassium thiocyanate solution

COLLABORATOR	SAMPLE $AgNO_3$	TITRATION CORR. TO 20°C.	NORMALITY AT 20°C.
	gram	*ml*	
H. W. Conroy,	0.7848	45.57	0.1014
F. & D. Administration,	.7097	41.18	.1014
Kansas City, Mo.	.7014	40.71	.1014
	.7325	42.51	.1014
H. C. Van Dame,	.6158	35.74	.1014
F. & D. Administration,	.6806	39.49	.1014
Cincinnati, Ohio	.6010	34.88	.1014
	.8265	47.99	.1014
	.7829	45.40	.1015
Mary E. Warren,	.7017	40.70	.1015
F. & D. Administration,	.7024	40.74	.1015
New Orleans, La.	.7000	40.57	.1016
	.7042	40.84	.1015
George Johnson,	.7034	40.82	.1014
F. & D. Administration,	.7121	41.34	.1014
St. Louis, Mo.	.7018	40.71	.1015
	.7101	41.21	.1014
C. L. Hoffpauir,	.6991	40.53	.1015
So. Regional Research	.6916	40.10	.1015
Lab., New Orleans, La.	.6946	40.29	.1015
	.6937	40.24	.1015
R. H. Robinson,	.7075	41.03	.1015
So. Regional Research	.7110	41.23	.1015
Lab., New Orleans, La.	.7055	40.91	.1015
	.7122	41.29	.1015
Jonas Carol,	.7288	42.41	.1012
F. & D. Administration,	.7112	41.33	.1013
Chicago, Ill.	.7581	44.01	.1014
	.7150	41.46	.1015
S. H. Perlmutter,	.7007	40.64	.1015
F. & D. Administration,	.7208	41.83	.1014
Minneapolis, Minn.	.7024	40.74	.1015
	.7008	40.67	.1014
	.7183	41.70	.1014
E. C. Deal,	.6908	40.10	.1014
F. & D. Administration,	.6905	40.09	.1014
New Orleans, La.	.7050	40.93	.1014
	.7134	41.36	.1015
	.6938	40.26	.1014

appear that the ammonium salt is to be preferred to the other salts for use in standard solutions. Water used in the preparation of thiocyanate solutions should be recently boiled. The solutions should be stored in clean bottles, preferably glass-stoppered.

It is recommended*—

(1) That the direct method of standardization of thiocyanate solutions with silver nitrate, as outlined in the revised procedure (p. 105) be adopted as official, first action.

(2) That this subject be closed.

REFERENCES

(1) *This Journal*, **24**, 44 (1941).
(2) *Ibid.*, **25**, 661 (1942).
(3) *Ibid.*, **28**, 595 (1944).

REPORT ON STANDARDIZATION OF POTASSIUM PERMANGANATE SOLUTIONS

By R. E. DUGGAN (Food and Drug Administration, Federal Security Agency, New Orleans 16, La.), *Associate Referee*

The official A.O.A.C. procedure has been previously reported[1] as yielding higher titers than the procedure[2] recommended by the National Bureau of Standards for use with their oxidimetric standard sodium oxalate. It has been shown that duplicable results may be obtained by either procedure and that the differences in the methods are significant only in the most exacting work.

For various reasons it has not seemed advisable to attempt to duplicate the work of Fowler and Bright[2] using other primary standards. In their procedure 90–95 per cent of the permanganate is added at room temperature and the titration completed at 55–60°C. This is a distinct handicap in working with unknown solutions since a preliminary titration must be made to ascertain the approximate amount of permanganate needed. The A.O.A.C. procedure titrates direct to the end point at 85°C. The purpose of this investigation is to determine whether the differences in the methods of titration are significant enough to warrant a change in the official method. In general, the standardization of solutions and the determination of an unknown should be carried out under as nearly identical conditions as possible. In actual practice, it should be possible to determine a substance as accurately by the A.O.A.C. procedure as by the National Bureau of Standards procedure using the respective standardization in each case.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 44 (1947).
[1] *This Journal*, **28**, 596–7, 1945.
[2] Fowler, R. M., and Bright, H. A., R.P. 843. *J. Res. Natl. Bur. Standards*, **15**, (1935).

Preliminary experiments tend to substantiate the above reasoning. Comparative results must be obtained on a number of different compounds using both procedures before a conclusion is justified. The differences are so small that, when ordinary volumetric equipment is used, a great deal of data is required to show that the variations are due to fundamental differences in the methods and are not due to experimental errors.

It is recommended* that the subject be continued.

REPORT ON BUFFER SOLUTIONS

By GEORGE G. MANOV (National Bureau of Standards, Department of Commerce, Washington, D. C.), *Associate Referee*

For the purpose of calibrating *p*H assemblies such as the hydrogen-calomel or the glass-calomel types, it is necessary to have on hand a number of salts of adequate purity from which buffer solutions of known *p*H can be prepared. The work described in a previous report[1] was therefore continued. A large number of measurements[2] of the effect of sodium chloride on the *p*H of borax buffers were made and permitted the certification of the *p*H values for these buffers in the absence of sodium chloride. The method used for the purification of the borax was presented in an earlier communication.[3] A 0.01–m solution of borax, 3.81 grams per liter, is recommended for use in the calibration of *p*H equipment.

Two additional salts, potassium dihydrogen phosphate and disodium hydrogen phosphate, intended for use together in equimolar proportions, have been purified by recrystallization from water to the extent that an error of less than 0.002 *p*H unit can be ascribed to residual impurities in the finished product.

The disodium salt separates from cold solutions as the dodecahydrate and cannot be dried directly to the anhydrous condition, because the former melts in its own water of crystallization at approximately 35°C and dries to form a hard cake of the heptahydrate. The heptahydrate can be dried to the anhydrous state directly. It has been generally recommended that the moist crystals be allowed to dry in air until the gross composition of mixture corresponds approximately to the heptahydrate, and to complete the drying in an oven. It was desired to obtain precise information concerning the amount of atmospheric carbon dioxide absorbed by such a moist, highly alkaline salt (*p*H = 9.3 at 25°C for the saturated solution) under conditions of protracted exposure to air and on the *p*H value for

* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 44 (1947).

[1] *This Journal*, **28**, 597 (1945).

[2] G. G. Manov, N. J. DeLollis, P. W. Lindvall, and S. F. Acree, *J. Research Natl. Bur. Standards*, **36**, 543 (1946) RP1721.

[3] G. G. Manov, N. J. DeLollis, and S. F. Acree, *J. Research Natl. Bur. Standards*, **33**, 287 (1944) RP1609.

the solution prepared from the exposed material. A study was therefore made of the relative purities of disodium hydrogen phosphate (a) recrystallized in the conventional manner and allowed to dry in air 6 weeks before final drying in an oven at 130°C, and (b) recrystalized in an atmosphere of nitrogen gas and the entire drying performed in a vacuum oven the temperature of which was gradually raised to 130°C. The carbon dioxide content of the two samples differed by only 0.01 per cent, and the *p*H values at 25°C for a solution having a buffer ratio of $Na_2HPO_4/KH_2PO_4 =$

TABLE 1.—*Recommended buffer solutions and pH values for calibration of pH equipment*[a]

TEMPERATURE, °C	0.05—m KH PHTHALATE	0.025—m KH_2PO_4 + 0.025—m Na_2HPO_4	0.01—m BORAX
0	4.012	6.983	9.463
5	4.005	6.950	9.389
10	4.001	6.922	9.328
15	4.000	6.896	9.273
20	4.001	6.878	9.223
25	4.005	6.860	9.177
30	4.011	6.849	9.135
35	4.019	6.842	9.100
40	4.030	6.837	9.066
45	4.043	6.834	9.037
50	4.059	6.833	9.012
55	4.077	6.836	8.987
60	4.097	6.840	8.961

[a] Preparation of solutions:
(1) Dissolve 10.21 g of potassium acid phthalate NBS Standard Sample 185 (dried for 1 hour a 105° C.) in sufficient distilled water to make 1 liter of solution.
(2) Dissolve 3.402 g of potassium dihydrogen phosphate and 3.549 g of disodium hydrogen phosphate NBS Standard Samples 186-I and 186-II (each dried for 2 hours at 130°C.) in sufficient distilled water to make 1 liter of solution.
(3) Dissolve 3.81 g of borax NBS Standard Sample 187 in sufficient distilled water to make 1 liter of solution.

2 agreed to within 0.002 *p*H unit. The conclusion was reached that air-drying of the moist crystals to the heptahydrate or below, followed by oven-drying, would not result in an unsatisfactory product. A number of other tests were performed, calculated to show the effects of small amounts of acidic or alkaline impurities on the *p*H of both the potassium dihydrogen phosphate and on the disodium hydrogen phosphate.

Twenty-five pound lots of each of the two phosphates and the borax were purified as above and were bottled[4] in portions of approximately 30 grams each. Solutions of these salts, and of potassium acid phthalate, can be used to calibrate *p*H equipment. The recommended concentrations

[4] The bottling was done by the Metals and Ore Analysis and Standard Samples Section of the National Bureau of Standards.

and *p*H values from 0° to 60°C for these buffers is given in Table 1.[5] The purification of additional standards suitable for use at other *p*H values is being continued.

The *p*H of the water used for the preparation of these solutions should be between 6.2 and 7.2 at 25°C.

It is recommended* that the studies on buffer solutions be continued.

REPORT ON STANDARD BROMIDE-BROMATE SOLUTION

By HALVER C. VANDAME (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

The work of the Associate Referee this year on the preparation and standardization of bromide-bromate solution was concerned with a preliminary study of three methods:

(1) Preparation of standard bromide-bromate solution by direct weighing of purified potassium bromate.

(2) Standardization of bromide-bromate solution against Bureau of Standards arsenious oxide.

(3) Standardization of bromide-bromate solution against standard thiosulfate solution.

PREPARATION OF STANDARD BROMIDE-BROMATE SOLUTION BY DIRECT WEIGHING

Analytical potassium bromate was recrystallized twice from water solutions and dried at 180°C. Exactly 5.5670 grams were weighed out and transferred to a two liter volumetric flask. Twenty-four grams of potassium bromide were added. Boiled distilled water was added and made to volume at 20°C. The calculated normality of this solution was 0.1000 *N*.

STANDARDIZATION OF BROMIDE-BROMATE SOLUTION AGAINST ARSENIOUS OXIDE

A standard arsenite solution was prepared by method given in "*Methods of Analysis*, 1945, **43.20**", using Bureau of Standards Sample No. 83. 2.4725 grams of arsenious oxide were weighed out and made to a volume of 500 ml at 20°C. The calculated normality of this solution was 0.1000 *N*.

METHOD

Measure 30 ml of the standard arsenite solution from a buret into a 300 ml Erlenmeyer flask. Add 10 ml of HCl and 3 drops of methyl orange T. S. Titrate the solution with the bromide-bromate solution prepared by direct weighing, constantly swirling during titration and adding the last ml dropwise. The end point is reached

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[5] A brief announcement of the availability and the cost of these standards appeared in *This Journal* **29**, 114 (1946).

* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 44 (1947).

when 1 drop of the bromide-bromate solution will cause the red color of the methyl orange to fade.

The results of this standardization are given in the following table:

TABLE 1.—*Standardization of bromide-bromate solution against arsenious oxide*

ARSENITE SOLN.	TEMP.	BROMIDE BROMATE SOLN.	TEMP.	ARSENITE SOLN. 20°C	BROMIDE BROMATE SOLN. 20°C	NORMALITY BROMIDE-BROMATE SOLN. 20°C
ml	°C.	*ml*	°C	*ml*	*ml*	
29.99	27	30.03	27	29.94	29.98	0.09987
29.99	27	30.03	27	29.94	29.98	0.09987
29.99	27	30.03	27	29.94	29.98	0.09987
29.99	27	30.04	27	29.94	29.99	0.09983
29.99	27	30.01	27	29.94	29.96	0.09993
29.99	27	30.01	27	29.94	29.96	0.09993
29.99	27	30.02	27	29.94	29.97	0.09990
29.99	27	30.02	27	29.94	29.97	0.09990

Av. Normality Bromide-Bromate Soln. at 20°C. =0.09989.

STANDARDIZATION OF BROMIDE-BROMATE SOLUTION AGAINST STANDARD THIOSULFATE SOLUTION

A standard solution of thiosulfate was used whose normality as checked by a standard iodine solution and by Bureau of Standards potassium dichromate was found to be 0.1004 *N*.

METHOD

Measure 30 ml of the standard bromide-bromate solution from a Bureau of Standards buret into an iodine flask. Add 25 ml of distilled water, 10 ml of a 10% KI solution, and 5 ml of HCl, stopper the flask and shake thoroly. Titrate the liberated iodine with standard thiosulfate solution, using starch solution as an indicator.

The results of this standardization are given in the following table.

TABLE 2.—*Standardization of bromide-bromate solution against standard thiosulfate solution*

BROMIDE BROMATE SOLN.	TEMP.	THIOSULFATE SOLN.	TEMP.	BROMIDE BROMATE SOLN. 20°C	THIOSULFATE SOLN. 20°C (1.1004 *N*)	NORMALITY BROMIDE BROMATE SOLN. AT 20°C
ml	°C	*ml of* (.1004 *N*)	°C	*ml*	*ml*	
29.99	27	29.83	27	29.94	29.78	0.09986
29.99	27	29.81	27	29.94	29.76	0.09980
29.99	27	29.83	27	29.94	29.78	0.09986
29.99	27	29.80	27	29.94	29.75	0.09976
29.99	27	29.80	27	29.94	29.75	0.09976
29.99	27	29.80	27	29.94	29.75	0.09976
29.99	27	29.81	27	29.94	29.76	0.09980
29.99	27	29.82	27	29.94	29.77	0.09983

Ave. Normality Bromide-Bromate Soln. at 20°C=0.09980.

CONCLUSIONS

The normality of a bromide-bromate solution prepared by direct weighing from recrystallized dried analytical grade potassium bromate agrees closely with the normality determined by titration against Bureau of Standards arsenious oxide and against a standard thiosulfate solution.

The indirect standardization of bromide-bromate solution against standard thiosulfate solution agrees well with the standardization against Bureau of Standards arsenious oxide; but there is a much greater chance for error and the procedure is much more time consuming than the direct standardization from the primary standard arsenious oxide.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative study be given to the standardization of bromide-bromate solutions against Bureau of Standards arsenious oxide.

(2) That the stability of bromide-bromate solutions be studied.

REPORT ON TITANIUM TRICHLORIDE SOLUTIONS

By Juanita E. Breit (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati 2, Ohio), *Associate Referee*

The work of the Associate Referee was concerned with a preliminary study on the preparation and standardization of titanium trichloride using existing methods as given below:

(1) Standardization of titanium trichloride using Method I, A.O.A.C., sixth edition, (1946) **21.37,** p. 290.

(2) Standardization of titanium trichloride using Method II (modified), *ibid.*

(3) Standardization of titanium trichloride using potassium dichromate and diphenylamine indicator.

PROCEDURE

Preparation of solution

150 ml of hydrochloric acid were added to 300 ml of commercial 15–16 per cent titanium trichloride (an old solution was used) and diluted to two liters. This solution was placed in a dark container with hydrogen atmosphere provision[1] and allowed to stand two days before using to permit the absorption of residual oxygen.

Method I

The procedure given in the *Methods of Analysis*[2] was used, substituting

* For report of Subcommittee A and action by the Association, see *This Journal*, **30**, 44 (1947).

[1] *Methods of Analysis, A.O.A.C.*, sixth edition (1945) figure 27, p. 289.

[2] *Ibid.*, Method I, **21. 37.**

iron wire for ingot iron in preparing the 0.1 N $Fe_2(So_4)_3$. The iron solution was standardized against 0.1 N $Na_2S_2O_3$ using KI and starch indicator.

0.1 N Fe_2 (So_4) 3—(F=1.010)	$TiCl_3$	NORMALITY
ml	*ml*	
40.0	46.4	0.08707
40.0	46.4	0.08707
40.0	46.4	0.08707

Method II

The procedure given in the *Methods of Analysis*[3] was used, substituting $K_2Cr_2O_7$ (Bureau of Standards) for $KMnO_4$.

0.1 N K_2 Cr_2 O_7	$TiCl_3$	NORMALITY
ml	*ml*	
40.0	47.00	0.08511
40.0	47.05	0.08502
40.0	47.05	0.08502
40.0	47.05	0.08502
40.0	47.10	0.08493

Method III

Titrate the titanium chloride solution in a strong stream of CO_2 against standard 0.1 N $K_2Cr_2O_7$ to which 50 ml of distilled H_2O, 5 ml of HCl and 5 drops of diphenylamine indicator is added. The indicator is prepared by dissolving 1.0 gm of diphenylamine in 100 ml of reagent H_2SO_4.

0.1 N $K_2Cr_2O_7$	$TiCl_3$	NORMALITY
ml	*ml*	
25.0	28.8	0.08681
25.0	28.8	0.08681
25.0	28.8	0.08681
25.0	28.7	0.08710

From the foregoing procedures it is impossible to state which method will prove most accurate and satisfactory. The Associate Referee prefers Method III, since it is quite simple and requires fewer chemicals, thus eliminating any errors due to impurities in the reagents.

It is recommended* that more work be done on the problem by submitting to collaborators samples of titanium trichloride for standardization, using the three methods as given above.

[3] *Ibid.*, Method II.

* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 44 (1947).

REPORT ON COSMETICS AND COAL-TAR COLORS

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

RECOMMENDATIONS*

Alkalies in Cuticle Removers.—No report received. Associate Referee recommends continuance but requests that problem be assigned to another Associate Referee. Referee concurs recommendation for continuance and recommends that another Associate Referee be appointed.

Cosmetic Creams.—C. F. Bruening reporting.

Cosmetic Powders.—No report or recommendations received from the Associate Referee. Referee recommends continuance.

Cosmetic Skin Lotions.—No report received. Associate Referee recommends continuance. Referee concurs.

Deodorants and Anti-Perspirants.—No report received. Associate Referee recommends continuance. Referee concurs.

Depilatories.—No report received. Associate Referee recommends continuance but does not wish to continue as Associate Referee. Referee concurs in recommendation and recommends that another Associate Referee be obtained.

Hair Dyes and Rinses.—No report received. Associate Referee recommends continuance. Referee concurs.

Hair Straightners.—No report received. Referee recommends continuance.

Mascara, Eyebrow Pencils, and Eye Shadow.—No report was received. Referee recommends that study be continued.

Mercury Salts in Cosmetics.—No report received. Associate Referee recommends continuance. Referee concurs.

Moisture in Cosmetics.—No report received. Associate Referee recommends continuance. Referee concurs.

Nail Cosmetics.—No report was received. Referee recommends that study be discontinued for the present.

Pyrogallol in Hair Dyes.—C. R. Joiner, Associate Referee, presents method and data from collaborative results. He recommends the adoption of method as tentative. Referee recommends method be adopted as official first action, for Pyrogallol in Hair Dyes not containing henna, and that the problem be continued.

Resorcinol in Hair Lotions.—F. M. Garfield, Associate Referee, presents method and reports data from collaborative results. He recommends that method be adopted as tentative. Referee recommends that method be adopted as official, first action, for Resorcinol in Hair Lotions.

Urea in Deodorants.—No report or recommendations received. Referee recommends that problem be discontinued.

* For report of Committee B and action by The Association, see *This Journal*, 30, 47 (1947).

Acetates, Carbonates, Halides, and Sulfates in Certified Coal-Tar Colors.—No report received. Associate Referee recommends continuance. Referee concurs.

Buffers and Solvents in Titanium Trichloride Titrations.—No report. The Associate Referee having retired, Referee recommends assignment to a new Associate Referee.

Ether Extract in Coal-Tar Colors.—No report received. Associate Referee recommends continuance. Referee concurs.

Halogens in Halogenated Fluoresceins.—No report. The Referee recommends continuance and assignment to a new Associate Referee.

Identification of Certified Coal-Tar Colors.—No report was received. Referee recommends continuance.

Intermediate in Certified Coal-Tar Colors.—The Referee recommends that problem be divided into the following subjects.

(1) Volatile Amine Intermediates
(2) Nonvolatile Unsulfonated Amine Intermediates
(3) Sulfonated Amine Intermediates
(4) Unsulfonated Phenolic Intermediates
(5) Sulfonated Phenolic Intermediates
(6) Intermediates Derived from Phthalic Acid.

As this problem is at present assigned, the Associate Referee is responsible for nearly 200 intermediates.

Mixtures of Coal-Tar Colors For Drug and Cosmetic Use.—No report received. Associate Referee recommends continuance. Referee concurs.

Lakes and Pigments.—K. A. Freeman reporting. Associate Referee recommends continuance. Referee concurs.

Spectrophotometric Testing of Coal-Tar Colors.—Rachel N. Sclar reporting. Associate Referee recommends continuance. Referee concurs.

Subsidiary Dyes in D&C Colors.—No report received. Associate Referee recommends continuance. Referee concurs.

The Referee recommends that the determination of lead in coal-tar colors be made a subject for study and an Associate Referee be assigned to this problem.

No report was given on alkalis in cuticle removers.

REPORT ON COSMETIC CREAMS

By CHARLES F. BRUENING (Food and Drug Administration, Federal Security Agency, Baltimore, Md.), *Associate Referee*

DETERMINATION OF GLYCEROL IN VANISHING CREAMS

In the Associate Referee's report of last year (1) a study was made of the Malaprade periodate method for the determination of glycerol in

vanishing creams. Briefly the procedure used isolated the glycerol and oxidized it by means of potassium periodate. Glycerol was oxidized by the periodate compound to formic acid, formaldehyde and water according to the following equation:

$$CH_2OH \cdot CHOH \cdot CH_2OH + 2KIO_4 \rightarrow HCOOH + 2HCHO + 2KIO_3 + H_2O$$

The quantity of glycerol involved was then ascertained by determining the amounts of periodate reduced and formic acid obtained in the oxidation. In that study on a vanishing cream containing a known amount of glycerol satisfactory results were obtained.

Following the recommendations in the previous report that the method as outlined be submitted to collaborative study, a sample of vanishing cream of the following composition was submitted to collaborators.

Composition of vanishing cream

	Per cent
Stearic acid	23.80
Water	63.48
Glycerol	11.48
Potassium hydroxide	1.13
Propyl p-hydroxy benzoate	.11

The proposed method used by the collaborators follows:

METHOD

REAGENTS

Potassium periodate—.02 *M*.—Dissolve 4.6 grams of C.P. KIO_4 in ca 500 ml of hot water. Dilute to about 900 ml with water, cool to room temperature, and make to 1 liter. This solution normally is approximately neutral to methyl red.

Potassium arsenite—.02 *N*.—Dilute 100 ml of U.S.P. 0.1 *N* potassium arsenite to 500 ml with water. In the preparation of the U.S.P. soln, if available, use Bureau of Standards, Standard Sample, Arsenious Oxide.

Sodium hydroxide—.02 *N*.—Use U.S.P. sodium hydroxide soln, fiftieth-normal.

Starch indicator.—Mix 0.5 gm of soluble starch with 10 ml of water and add 90 ml of boiling water. Heat to boiling for ca 5 min.

Methyl Red indicator solution.—Use U.S.P. Test Soln.

PROCEDURE

Isolation of glycerol.—Place 2 to 10 gm of sample in a separatory funnel, add 25–50 ml of water, acidify slightly with dilute H_2SO_4 (10 gm/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. Transfer the aqueous soln and the wash water to a 250 ml volumetric flask, add 2 drops of methyl red indicator soln and 0.1 *N* NaOH soln until the soln is neutral, and then dilute to mark with water.

Periodate oxidation.—Transfer an aliquot of the neutral soln preferably containing 30–40 mg of glycerol to a 100 ml volumetric flask and add 50 ml of .02 *M* KIO_4. Make to mark with water and allow to stand ca 1 hour.

Formic acid titration.—Transfer a 50 ml aliquot of the oxidized mixture to a titration flask. Add 2 drops of methyl red indicator soln and titrate with .02 *N* NaOH to a definite clear yellow.

Apply an appropriate correction for any acidity in the .02 *M* KIO_4, by titrating a 25 ml sample of the KIO_4 soln with .02 *N* NaOH, using methyl red indicator soln.

1 ml of .02 *N* NaOH = 1.84 mg of glycerol.

Potassium periodate determination.—Transfer a 20 ml aliquot to a titration flask and dilute with ca 50 ml water. Add ca 1.0 gm of $NaHCO_3$, 0.5 gm of KI, and 5 ml of starch indicator. Titrate immediately with the .02 *N* $KAsO_2$ soln to the disappearance of the blue color.

Standardize 10 ml of the .02 *M* KIO_4 by this same titration procedure.

The difference between the two titrations represents the amount of periodate reduced in the 20 ml aliquot taken. To obtain the amount of periodate reduced in the original aliquot obtained from the 250 ml flask, it is necessary to multiply the above difference by 5.

1 ml of .02 *N* $KAsO_2$ = 0.46 mg of glycerol.

In using this method the collaborators were instructed to use ca 2 gm of the vanishing cream as the original sample. The glycerol content of the cream (11.48 per cent) was such that aliquots selected from the 250 ml flask for oxidation, in order to fall in the range of 30 to 40 mg of glycerol as required by the method, should represent not less than 0.27 gm or more than 0.35 gm of the original sample. With exactly 2 gm of sample, aliquots 35 to 45 ml were suggested.

This range was originally selected in the method for convenience. If smaller amounts than 30 mg of glycerol were used the titrations became too small and materially affected the accuracy and precision possibilities of the method. If amounts much in excess of 40 mg of glycerol were used, the 50 ml potassium periodate solution would be insufficient for complete oxidation.

Each collaborator was requested to calculate the amount of glycerol by the formic acid determination with and without the benefit of the correction for the acidity of the potassium periodate solution.

In the potassium periodate determination each collaborator was instructed to standardize the potassium periodate solution because this solution is subject to decomposition and decreases in strength on standing.

The results obtained by the various collaborators are given in Table 1.

COLLABORATORS

(1) M. Matluck, U. S. Food and Drug Administration, Boston, Mass.

(2) S. H. Newburger, Cosmetic Division, U. S. Food and Drug Administration, Baltimore, Md.

(3) Shirley M. Walden, U. S. Food and Drug Administration, Baltimore, Md.

(4) K. H. Ferber, National Aniline Division, Allied Chemical and Dye Corporation, Buffalo, N. Y.

(5) Harry Isacoff, U. S. Food and Drug Administration, New York, N. Y.

(6) Edward C. Fearns, Lever Bros. Co., Cambridge, Mass.

(7) N. E. Freeman, U. S. Food and Drug Administration, Atlanta, Ga.

(8) Louis B. Dobie, Bristol-Myers Co., Hillside, N. J.

(9) C. F. Bruening, U. S. Food and Drug Administration, Baltimore, Md.

TABLE 1.—*Recovery of glycerol in vanishing cream (11.48% glycerol)*

COLLABORATOR	GLYCEROL FOUND FORMIC ACID DETERMINATION (UNCORRECTED)	GLYCEROL RECOVERY (AVE.)	GLYCEROL FOUND FORMIC ACID DETERMINATION (CORRECTED)	GLYCEROL RECOVERY (AVE.)	GLYCEROL FOUND PERIODATE DETERMINATION	GLYCEROL RECOVERY (AVE.)
No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	11.12		11.01		11.50	
	11.14	97.0	11.04	96.0	11.50	100.2
2	11.37		11.32		11.50	
	11.34	98.9	11.28	98.4	11.56	100.4
3	11.07		10.94		11.73	
	11.22	97.1	11.09	95.9	11.75	102.3
4	11.2		11.2		11.6	
	11.2	97.6	11.1	97.1	11.6	101.0
5			10.96		11.44	
			11.05		11.47	
			11.05			
			11.14	96.3		99.8
6	10.88		10.67		11.24	
	10.88		10.67		11.24	
	11.10	95.4	10.90	93.6	11.28	
					11.28	98.1
7			10.80		11.28	
			10.54		11.24	
			10.74	93.1	11.25	98.1
8	11.46	99.8	11.46	99.8	11.43	99.6
9	11.24		11.14		11.52	
	11.29	98.1	11.18	97.2	11.53	100.4
Ave.	11.22	97.7	11.07	96.4	11.48	100.0

DISCUSSION OF RESULTS

The results given in Table 1 show that low recoveries were obtained for glycerol in both formic acid determinations, the first in which the acidity of the potassium periodate solution was uncorrected and the second in which a correction was made for this factor. However, the differences between the corrected and uncorrected results are comparatively small, the collaborators reporting that the correction used varied from .05 to .30 ml .02 *N* sodium hydroxide solution for 25 ml of the .02 *M* potassium periodate solution.

It is believed (2) that low recoveries for glycerol are obtained by the formic acid method because of the characteristics of the methyl red indicator in periodate solutions.

Formic acid is a weak acid and when titrated with alkali the equivalent point is on the basic side. Thus, a basic indicator such as phenolphthalein would be preferable, but this indicator cannot be used in the presence of the excess periodate because KIO_4 titrates as an acid. Actually, in the formic acid titration using methyl red indicator a very gradual color change was noted as the end point was reached. To approach the basic side of the solution as closely as possible it was suggested in the method that a definite clear yellow end point should be obtained.

In determining the acidity correction it was also noted that when the potassium periodate solution was titrated with alkali, small amounts—from .05 to .30 ml of .02 *N* sodium hydroxide—were needed to change the methyl red indicator from the usual red to a light salmon color. On further addition of alkali it was impossible to obtain a definite titration which resulted in a clear yellow end point of the same color as that obtained in the formic acid titration.

Thus, in the actual determination of glycerol by the formic acid titration, the periodate solution had to be titrated to a salmon end point while the formic acid solution had to be titrated to a clear yellow end point. This variance in the color of the two end points suggests that methyl red may not be altogether satisfactory as an indicator.

One of the collaborators, Edward C. Fearns, in addition to submitting results by the proposed formic acid titration, also determined glycerol by a somewhat similar method as given by Bradford, Pohle, Gunther, and Mehlenbacker (3). In this method the oxidizing agent, periodic acid, is titrated potentiometrically, using 0.125 *N* sodium hydroxide to *p*H 5.40, and the solution containing the formic acid to *p*H 6.20. The difference between the two titrations is a measure of the amount of glycerol present. Prior to the addition of the periodic acid, the *p*H of the glycerol solution is brought to 6.20. This method gave an average glycerol content of 11.27 per cent or 98.2 per cent recovery. These results are somewhat higher than the average for all collaborators and tend to substantiate the belief that low recoveries were obtained in the formic acid titration because of the limited suitability of methyl red as the indicator.

The formic acid determination was studied in the hope that the results would assist in identifying glycerol as contrasted to other glycols used in cosmetic creams which do not yield formic acid on oxidation. Thus, if the amount of glycerol found by the formic acid titration is the same as that found by the periodate reduction determination, the presence of glycerol alone is qualitatively indicated. It is believed that the above results fulfill this qualitative indication of glycerol in this cream, but it is apparent that the formic acid determination is less accurate than the periodate determi-

nation. Further study of the formic acid titration for the determination of glycerol is contemplated.

The results for glycerol by the potassium periodate determination obtained by all the individual collaborators are satisfactory. The recoveries all fall in the range of 100 per cent ± 2.3 per cent. The average recovery is also satisfactory. Apparently, this method offered no difficulties to any of the collaborators, suggesting, when considered with the recoveries, that the determination of glycerol by this method is accomplished with ease and accuracy.

ACKNOWLEDGEMENT

The Associate Referee expresses his gratitude to the collaborators for their generous efforts.

RECOMMENDATIONS*

It is recommended—

(1) That the method for glycerol in vanishing creams be submitted to further study.

REFERENCES

(1) *This Journal.* **29**, 29 (1946).
(2) S. H. NEWBURGER, Private Communication.
(3) *Oil and Soap*, **19**, 189–93 (1942).

No reports were made on the following subjects: cosmetic powders, cosmetic skin lotions, deodorants and anti-perspirants, depilatories, hair dyes and rinses, hair straighteners.

No reports were made on mascara, eyebrow pencils, and eye shadow, or on mercury salts in cosmetics, moisture in cosmetics, or nail cosmetics.

REPORT ON PYROGALLOL IN HAIR DYES

By CURTIS R. JOINER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In a previous report[1] the Associate Referee presented a method for the determination of pyrogallol in liquid hair dyes and the results of collaborative study with the method. He recommended that further work be done on the applicability of the proposed procedure. The published method was a revision of the one that had been sent to the collaborators, and some further changes were made before it was again subjected to collaborative study. The original method was not applicable to any liquid preparation

* For report of Subcommittee B and action of the Association, see *This Journal*, **30**, 47 (1947).
[1] *This Journal*, **28**, 744 (1945).

that might contain chlorophyll. As a result of work done on henna powder-pyrogallol mixtures, a provision has been made in the present method for the elimination of chlorophyll as an interference through the use of alumina cream as a clarifying agent. Some recent work has shown that with the particular instrument in use in this laboratory the standard curve is not a straight line below a concentration of one milligram per 100 ml. That this may not be true with all photometers is indicated by the fact that the majority of the collaborators drew their standard curves as a straight line.

For details of the method, see under "Changes in Methods of Analysis," *This Journal*, **30**, 61 (1947).

COLLABORATIVE WORK

Two samples similar in composition to the ones sent out in 1944 were again sent to collaborators. These samples were prepared as follows:

No. 1

Pyragallol	37.500 grams
Sodium bisulfite	25.000 grams

Dissolved in distilled water and diluted to one liter.

No. 2

Pyrogallol	30.000 grams
Sodium bisulfite	40.000 grams
Nickelous sulfate	50.000 grams
Ferric chloridè	25.000 grams

Dissolved in distilled water and diluted to one liter.

The collaborators were requested to make duplicate determinations on both samples, using either of the alternate methods of extraction. The results are summarized in Table 1.

STATISTICAL INTERPRETATION OF RESULTS

Sample 1: Standard deviation, ±0.059 g., or ±1.6%; probable error of a single determination, ±0.040 g., or ±1.1%.

Sample 2: Standard deviation, ±.038 g., or ±1.3%; probable error of a single determination, ±.026 g., or ±0.88%.

COLLABORATORS' COMMENTS

Braun.—"No particular difficulty was encountered in the analysis. Since the shakeout method proved just as efficient as the continuous extractor I would prefer the former."

Van Dame.—"I encountered no difficulty in following directions."

Moraw.—"Suggest two or three ether extractions be made and used after the 6th for a test for complete extraction."

Perlmutter.—"The solutions after color development showed negligible change after one hour by Klett colorimeter. In the method, I suggest the word 'immediately' be replaced by 'within 30 (or 15) minutes.' This would allow a reading of a series of samples a few minutes apart.

TABLE 1.—*Collaborative results*

COLLABORATOR	PYROGALLOL RECOVERY				METHOD OF EXTRACTION[a]
	SAMPLE 1		SAMPLE 2		
	g./100 ml.	*Per cent*	*g./100 ml.*	*Per cent*	
1	3.77	100.5	2.94	98.0	2
	3.76	100.3	2.98	99.3	1
2	3.78	100.8	2.99	99.7	2
	3.80	101.3	3.02	100.7	1
3	3.71	98.9	2.93	97.7	2
	3.71	98.9	2.93	97.7	2
4[b]	3.74	99.7	2.93	97.7	2
	3.75	100.0	2.94	98.0	2
5[c]	3.82	101.9	2.96	98.7	1
	3.80	101.3	2.98	99.3	1
6	3.75	100.0	3.00	100.0	2
	3.75	100.0	2.96	98.7	2
7[b]	3.64	97.1	2.81[f]	93.7	2
	3.61	96.3	2.87	95.7	2
	3.67	97.9	2.96	98.7	1
	3.69	98.4	2.93	97.7	1
8[b]	3.57[f]	95.2	2.80[f]	93.3	2
	3.62	96.5	2.87	95.7	2
	3.65	97.3	2.95	98.3	1
	3.67	97.9	2.91	97.0	1
Associate	3.76	100.3	2.96	98.7	2
Referee	3.77	100.5	2.99	99.7	1[d]
	3.75	100.0	2.98	99.3	1[e]
Averages	3.73	99.5	2.95	98.3	

[a] (*1*) continuous extractor; (*2*) separatory funnels.
[b] Used an American Instrument Co. neutral wedge photometer; all other analysts used the neutral wedge photometer described by Clifford and Brice.[2]
[c] Readings were made with the same solutions in a Klett photoelectric colorimeter, using filter No. 54 (No. 56 was not available). Results: Sample 1, 3.80 (3.80) g./100 ml; Sample 2, 2.96 (2.96) g./100 ml.
[d] Continuous extractor of approximately 15 ml capacity.
[e] Palkin extractor of approximately 50 ml capacity. Samples diluted to 50 ml with water.
[f] Not included in the averages. Statistical analysis showed that according to the Pierce-Chauvenet criterion[3] these results could be discarded.

"Sample 2 turned black after ether evaporated.

"There were no difficulties encountered; the instructions are adequate."

Thomas.—"In the case of Sample 2, extremely rapid darkening of the solution

[2] Clifford, P. A., and Brice, B. A., *Ind. Eng. Chem., Anal. Ed.*, 12, 218 (1940).
[3] Crumpler, Thomas B., and Yoe, John H., *Chemical Computations and Errors* (New York, 1940), pp. 189–190.

resulted on placing it in the separatory funnel even with sodium bisulfite present . . . "

Conroy.—"Slightly higher results by continuous extraction may be accounted for by the fact that no loss of aqueous layer was sustained. It was noted that in the shakeout with separatory funnels, a few drops of the aqueous layer were carried along with the ether layer and were retained on the cotton pledget."

Bennett.—"Another observation made was that readings on the same sample gave lower results on the upper part of the curve . . . "

DISCUSSION

On consideration of the above comments two minor changes in the method were made. The instructions regarding the test for complete extraction with separatory funnels were modified to permit the analyst to make one or two additional extractions as he sees fit. The directions for making photometer readings after development of the color were changed from "immediately" to "within ten minutes." Perlmutter submitted some data on this point which showed only small changes in readings for several solutions over a period of one hour. These results were not far different from those obtained by the author. This change was made to allow a practical interval of time for the analyst to complete the readings.

One collaborator who used continuous extractors commented that Sample 2 turned black after the ether extract was evaporated. This was caused by a reaction between the pyrogallol and the small amounts of iron and cobalt salts carried over into the extraction flask during the period of the extraction. In analyzing samples of this type the author has had the solution to darken slightly sometimes but never to an extent that would affect the final result.

Bennett stated that he obtained lower results by taking readings on the upper part of the curve than he got on the same sample by using an aliquot that gave lower readings. Apparently, none of the other collaborators had this difficulty since no one mentioned it. However, the author has frequently made readings with different sized aliquots from the same sample, and the differences have been random and within the error of the method.

Analysts 7 and 8 first reported duplicate determinations that were lower than those of the other collaborators. They were asked to repeat the analyses on additional samples, and their second set of results were slightly higher than the first ones. All of their determinations are listed in the table. However, three of the first results are not included in the averages since a statistical analysis showed that they could be discarded.

SUMMARY AND RECOMMENDATIONS

The analytical results reported above are better than the ones obtained in 1944. A statistical analysis shows that 50 per cent of determinations on samples similar to those used in this study can be expected to fall within about plus or minus one per cent of the mean. The slightly low value

of the average for Sample 2 can be attributed to the fact that pyrogallol is readily decomposed in solution with metallic salts.

It is recommended* that the method be adopted as tentative for the determination of pyrogallol in liquid hair dyes, and that further work be done on the extraction of pyrogallol from solid hair dyes.

EXTRACTION OF PYROGALLOL FROM HENNA POWDER-PYROGALLOL MIXTURES

The only two promising solvents investigated for the extraction of pyrogallol from henna powder mixtures were ethyl acetate and ethyl ether. Of these two the former was found to be superior because of its higher boiling point and because it dissolved the pyrogallol from the mixture more rapidly than did ethyl ether.

Two methods of extraction were tried. In the first one a weighed sample was transferred to a ground glass-stoppered Erlenmeyer flask, a measured volume of solvent added, and the flask shaken for one hour. After standing for approximately 24 hours the solution was filtered and an aliquot of the filtrate used for determining the pyrogallol. This method gave 100 per cent recoveries on several freshly prepared mixtures, but as the samples aged recoveries gradually decreased. On one three-months old mixture a 67 per cent recovery was obtained by shaking the sample with the solvent for four 8-hour days.

The second method consisted of extracting a weighed sample in a Soxhlet extractor for several hours. Here again, recoveries of approximately 100 per cent were obtained on freshly prepared mixtures. However, it was necessary to connect drying tubes to the reflux condensers, since it was found that water condensing on the inside of the apparatus gave high blanks on the henna powder used to prepare the mixtures. With this precaution the blanks were reduced to a maximum of 0.25 per cent, or about five per cent of the amount of pyrogallol in the mixtures. For an 8-hour extraction, recoveries from 90 to 95 per cent were obtained on mixtures that were several months old, and additional extractions gave no further recoveries of pyrogallol.

One problem that was common to both methods of extraction was the separation of the extracted pyrogallol from chlorophyll. This separation was accomplished by evaporating the ethyl acetate solution to dryness, taking up the residue in water, adding two ml of alumina cream, diluting to a definite volume and filtering the mixture. This treatment resulted in a clear solution and caused no loss of pyrogallol. The clear solution was used for the determination of pyrogallol by the proposed colorimetric method.

The extraction with continuous extractors should be investigated further to determine the following factors:

* For report of Subcommittee B and action of the Association, see *This Journal*, 30, 47 (1947).

(1) The optimum conditions for making the extraction and the maximum recovery obtainable from mixtures several months old.

(2) The conditions which will give the minimum blank.

(3) The relative efficiency of the Soxhlet and the Palkin-type extractors.

ACKNOWLEDGEMENT

The author wishes to express his appreciation to the following members of the Food and Drug Administration who took part in this work as collaborators: S. H. Perlmutter, Minneapolis, Minn.; H. W. Conroy and H. P. Bennett, Kansas City, Mo.; J. A. Thomas, New Orleans, La.; M. A. Braun and Mary A. McEniry, St. Louis, Mo.; H. O. Moraw, Chicago, Ill.; H. C. Van Dame, Cincinnati, Ohio.

REPORT ON RESORCINOL IN HAIR LOTIONS

By FREDERICK M. GARFIELD (U. S. Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

In the determination of resorcinol in hair lotions the analysis resolves itself into two operations, (1) isolation of the resorcinol, and (2) quantitative estimation of the isolated material. In the last report of the Associate Referee[1] a satisfactory isolation procedure was reported and two quantitative methods for resorcinol suggested. The first method consisted of a direct nitrosation of resorcinol to 2,4-dinitrosoresorcinol in acid solution, by means of sodium nitrite. The second method was the well known bromination reaction. As was pointed out at that time, the nitrosation had the advantage over the bromination in that it was more specific for resorcinol. However, the recoveries obtained by the nitrosation procedure were not quantitative, ranging from 92 to 99 per cent.

Work last year, for the most part, was centered on the nitrosation method. The usual temperature ranges, concentrations, times, acids, etc., were investigated, but the results obtained were either not accurate or not precise. In addition, titrations were made with standard sodium nitrite, in acid solution, using starch-iodide as an outside indicator. Nitrosation was rapid in the early stages of the titration, but slowed down as the end point was approached. The final end point was much too indefinite for a quantitative procedure. For these reasons the nitrosation method was reluctantly dropped.

The bromination of resorcinol gave excellent recoveries on pure solutions and on resorcinal recovered from prepared hair lotions. In the bromination method an excess of bromide-bromate solution was added to the resorcinol, the solution was made acid and the bromination was allowed to proceed for one minute. Potassium iodide was added and the iodine liberated by the excess bromine was titrated with thiosulfate, using starch indicator.

[1] *This Journal*, **25**, 899 (1942).

In the bromination of various materials a certain excess of bromine must be present to obtain quantitative results. In the case of resorcinol the bromination proceeds rapidly and only a small excess of bromine is required. This can be illustrated by a set of results obtained during the investigation.

1.5000 g of USP resorcinol was made to 500 ml with water. Aliquots from 5–30 ml were taken for analysis, 50 ml of tenth normal bromine being added to each.

RESORCINOL ALIQUOT	N/10 BROMINE REACTING	RESORCINOL RECOVERED
ml	*ml*	*grams*
5	8.11	1.488
10	16.34	1.499
20	32.58	1.495
25	40.56	1.489
30	47.25	1.444

PREPARATION OF HAIR LOTION FOR COLLABORATORS

It is obvious that a hair lotion used in control work should contain as many as possible of the most commonly used ingredients. Accordingly, a solution was prepared for collaborative study containing more than the usual number of ingredients ordinarily present in any commercial hair lotion.

Ingredients	*Grams*	*Ingredients*	*Grams*
Resorcinol (Assaying 99.52% resorcinol by the method given below)	10.4000	beta-Naphthol	10.0
		Quinine sulfate	2.0
		Glycerin	20.0
Boric acid	10.0	Alcohol	400 ml.
Salicyclic acid	5.0	Water to make	1000 ml.
Chloral hydrate	5.0		

Based on a purity of 99.52 per cent (ave. 4 analyses) the lotion contained 1.035 g resorcinol per 100 ml. This solution was submitted to the collaborators. Results and comments are given below.

The method is given under "Changes in Methods of Analysis," in *This Journal*, **30**, 62 (1947).

RESULTS

Collaborators reported the following recoveries. (See next page).

COMMENTS OF COLLABORATORS

S. H. Newburger.—(1) Couldn't the term "dealcoholize" be made more specific by adding a qualifying phrase "dealcoholize by evaporating to ——ml"? (2) It might be helpful to the analyst if a note were added that most of the solid material

COLLABORATOR	RESORCINOL	RECOVERY
	g./100 ml.	*per cent*
1) G. M. Johnson, St. Louis, Mo.	1.012	97.8
	1.017	98.3
	1.018	98.4
2) A. W. Hanson, Minneapolis, Minn.	1.030	99.5
	1.038	100.3
3) S. H. Newburger, Washington, D. C.	1.025	99.0
	1.022	98.7
4) H. C. Van Dame, Cincinnati, Ohio	1.026	99.1
	1.026	99.1
	1.027	99.2
5) R. D. Stanley, Chicago, Ill.	1.043	100.8
	1.047	101.2
6) H. Bond, Kansas City, Mo.	1.022	98.7
	1.024	98.9
	1.025	99.0
7) F. M. Garfield, St. Louis, Mo.	1.029	99.4
	1.026	99.1
Average	1.027	99.2

remaining after dealcoholization is transferred by the chloroform rather than the water washing.

G. M. Johnson.—The titration of the excess bromine by iodine and sodium thiosulfate seems to be a critical point in this determination. Even if the titration is rapid after the addition of the KI, there is still reason to question the accuracy of the results due to the aerial oxidation of the HI in the presence of HCl. It is suggested that the amount or concentration of the acid be reduced either by adding less during the actual bromination or by considerable dilution of the solution before titration. In the standardization of $Na_2S_2O_3$ by $K_2Cr_2O_7$ only 20 ml of normal HCl are used in a total volume of 100 ml.

Other collaborators made no comment or stated they had no difficulty with the method.

DISCUSSION

The note suggested by Newburger should be added to the method.

The difficulty suggested by Johnson was not encountered or at least not noticed during the study. It will be investigated by the Associate Referee.

RECOMMENDATIONS*

It is recommended (1) that the method outlined be adopted as a tenta-

* For report of Subcommittee B and action of the Association, see *This Journal*, 30, 48 (1947).

tive method for the quantitative determination of resorcinal in hair lotions and (2) that the procedure be subjected to further collaborative study.

No reports were made on the following subjects: urea in deodorants; acetates, carbonates, halides, and sulfates in certified coal-tar colors; buffers and solvents in titanium trichloride titrations; ether extract in coal-tar colors; halogens in halogenated fluoresceins; identification of certified coal-tar colors; intermediates in certified coal-tar colors (see Referee report); and mixtures of coal-tar colors for drug and cosmetic use.

REPORT ON LAKES AND PIGMENTS

D&C RED No. 8

By KENNETH A. FREEMAN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Section **21.41(d)**, *Methods of Analysis, A.O.A.C.*, 6th Ed., gives a method for the determination of pure dye in D&C Red No. 8 by direct titration with standard titanium trichloride solution.

In order to determine the accuracy of the method, it was decided to prepare D&C Red No. 8 as nearly 100 per cent pure as possible, analyze it by several different methods, and compare the results obtained.

EXPERIMENTAL

(A) *Purification of 2-chloro-5-toluidine-4-sulfonic acid (Lake Red C Amine)*: 100 grams commercial Lake Red C Amine paste was suspended in 500 ml of water, heated to near boiling and 10 per cent NaOH solution slowly added until practically all material was in solution. 5 grams of activated charcoal were added and the mixture was boiled for 5 minutes, then filtered hot through a steam-jacketed Büchner funnel. The filtrate was cooled in an ice bath with constant stirring, then filtered immediately. The precipitated crystals were washed twice with 25 ml of cold distilled water and dried at 135°C. The product was a pure white material crystallized in small shiny plates. By analysis for Cl—100.5 per cent Lake Red C Amine sodium salt.

(B) *Preparation of Lake Red C*: 10 grams of the Lake Red C Amine sodium salt obtained above was dissolved in 700 ml distilled water. While stirring mechanically, 2 ml of concentrated HCl were added drop by drop to form a finely divided suspension of Lake Red C Amine. The suspension was cooled to 5°C., and 8.5 ml of concentrated HCl and 98 ml of a 10 per cent $NaNO_2$ solution cooled to 5°C, were added. The mixture was held at 5°C for one hour while stirring constantly. The suspension of the resulting diazo compound was then added to a cold (5°C.) solution of 6.5 grams pure b-naphthol, 2 grams NaOH, and 4.5 grams Na_2CO_3 in 500 ml of

water. The mixture was stirred continuously for two hours at 5°C., then removed from the cooling bath and allowed to stand at room temperature overnight. The precipitated Lake Red C was filtered off, suspended in 500 ml of approximately 0.1 *N* NaOH, boiled for 2 minutes, and filtered while hot. The material on the filter was washed with two 25 ml portions of distilled water. The precipitate was then suspended in 200 ml ethyl alcohol containing 1 ml concentrated HCl, brought to a boil, filtered while hot and washed with 25 ml of cold alcohol, followed by several small portions of ether. The product was dried at room temperature, pulverized and dried in an oven at 135°C for 24 hours. Yield: 15 grams (91 per cent of theoretical).

(C) *Analysis of Lake Red C*: The results of analyses of the material are summarized in the following table:

	FOUND	THEORETICAL FOR PURE DYE	PURE DYE BY CALC.
	per cent	*per cent*	*per cent*
Pure Dye by—			
Titration ($TiCl_3$)	98.9		
Nitrogen	6.92	7.02	98.6
Sulfur	7.95	8.04	98.9
Chlorine	8.78	8.89	98.8
Sodium	5.68	5.76	98.6

From these results it was obvious that some material not accounted for by any of these determinations was present. A portion of the color was then dried for three hours over phosphorus pentoxide in an Abderhalden drier held at the temperature of boiling toluene. The system was evacuated by a Hyvac pump to 2 mm of mercury. The loss in weight was 1.3 per cent. Titrations of the dried material showed 100.1 per cent, 100 per cent, and 99.5 per cent pure dye present.

CONCLUSION

As a result of this investigation it is concluded that the titration procedure can be expected to give satisfactory results in the analysis of Lake Red C.

It is recommended* that work on the topic "Lakes and Pigments" be continued.

* For report of Subcommittee B and action of the Association, see *This Journal*, 30, 48 (1947).

REPORT ON SPECTROPHOTOMETRIC TESTING

SPECTROPHOTOMETRIC ANALYSIS OF COAL-TAR COLORS, D&C RED NO. 35, D&C RED NO. 36

By RACHEL N. SCLAR (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

This report presents a continuation of the investigation of spectrophotometric analysis of dyes certifiable under the Coal-Tar Color Regulations.[1] The spectrophotometric method of analysis of colors that obey Beer's law in their solutions permits a quantitative determination of the pure dye content as well as identifying the dye. The method is especially useful for dyes whose purity previously was best evaluated from the nitrogen content.

As in the previous investigation,[2] dyestuffs of high purity were prepared to serve as standards. The data obtained from the spectrophotometric analysis of solutions of these dyes were used to establish the following:

1. Conformity of the color to Beer's law.
2. Location of the absorption peaks.
3. The ratio of the extinction coefficients at specific wave lengths, chosen on opposite sides of the maximum absorption peak, where possible, as a further means of identifying the dye.[3]

Measurements were made with a General Electric recording spectrophotometer equipped with automatic slit adjustment for an 8 millimicron wave length band.

Results obtained in the investigation of D&C Red No. 35 (Toluidine Red) and D&C Red No. 36 (Flaming Red) are given in this report.

D&C RED NO. 35

Experimental

Preparation of Standard Dyestuff.—3-nitro-4-aminotoluene (m.p. 117°C) was diazotized and coupled with β-naphthol (m.p. 116–117°C—on Fisher block) in alkaline solution. The product was crystallized twice from chloroform; it melted at 276.5°C (literature[4] m.p. 278°C). A separate synthesis of this dye followed by crystallization from 2-nitropropane gave a product having the same melting point.

Spectrophotometric examination of chloroform solutions of dye prepared from each of these two batches showed no difference in the extinction data. The material crystallized from chloroform was therefore considered sufficiently pure to serve as a standard.

Preparation of Solutions.—A 16.52 mg portion of dye was weighed on a semimicro balance, sensitive to 0.02 mg, and dissolved by refluxing with

[1] U. S. Food and Drug Administration, Service and Regulatory Announcements, F. D. C. 3.
[2] G. R. Clark and S. H. Newburger, "Spectrophotometric Analysis of Coal-tar Colors I, Ext. D&C Yellow No. 5," *This Journal*, 27, 576 (1944).
[3] U. S. Department of Agriculture, Technical Bull. No. 310, June 1932, p. 21.
[4] Gustav Schultz, Farbstoff Tabellen, 7th Edition, Volume I, p. 47.

50 ml of U.S.P. chloroform. The solution was cooled to room temperature, transferred to a 100 ml volumetric flask, and made to volume with chloroform. Aliquot portions of this solution were diluted with chloroform to the concentrations shown in Table 1. All solutions were made to volume at the temperature of the room in which the optical measurements were made.

The stability of a solution prepared as above was determined by aging for a period of 4 hours. Spectrophotometric curves drawn hourly showed that no fading of color occurred.

TABLE 1.—*Extinction values of solutions of D&C Red No. 35 in U.S.P. chloroform*

Typical Data

CURVE NO. (CHART 1)	CONCENTRATION MG./LITER	EXTINCTION			$E_{511\ m\mu}$ CONCENTRATION	$\frac{E_{500\ m\mu}}{E_{520\ m\mu}}$
		500 mμ	511 mμ	520 mμ		
1	2.06	.152	.156	.142	.0757	1.070
2	4.13	.302	.312	.286	.0755	1.056
3	8.26	.604	.622	.573	.0753	1.054
4	16.52	1.206	1.244	1.144	.0753	1.054
					av. .0754	1.06

Spectrophotometric Data.—The extinction curves for the chloroform solutions of D&C Red No. 35 are shown in Chart 1. They show an absorption peak at 511 ± 2 mμ. (All wave lengths were corrected to ± 2 mμ with the aid of didymium glasses tested by the National Bureau of Standards; see footnote to Charts 1–4, inclusive.)

The ratio of extinction values at 500 and 520 mμ was calculated. This ratio, $E_{500\ m\mu}/\ E_{520\ m\mu} = 1.06 \pm .01$; see Table 1.

DISCUSSION

The ratios of extinction to concentration in Table 1 indicate that at 511 mμ, chloroform solutions of D&C Red No. 35 containing 2 to 16.5 mg of color per liter conform to Beer's law. The pure dye content of a sample of this color can therefore be determined by dissolving a weighed portion of the sample in chloroform and obtaining spectrophotometric data from the solution.

APPLICATION TO COMMERCIAL SAMPLES

Primary Colors and Lakes

Two samples of certified D&C Red No. 35 and one sample of a [illegible] Red No. 35 Lake (made by extending the dye on insoluble subs[illegible] were analyzed spectrophotometrically. Weighed samples were r[illegible] with chloroform to dissolve the color. With lakes, filtering the s[illegible]

50 ml of U.S.P. chloroform. The solution was cooled to room temperature, transferred to a 100 ml volumetric flask, and made to volume with chloroform. Aliquot portions of this solution were diluted with chloroform to the concentrations shown in Table 1. All solutions were made to volume at the temperature of the room in which the optical measurements were made.

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APPLICATION TO COMMERCIAL SAMPLES

Primary Colors and Lakes

Two samples of certified D&C Red No. 35 and one sample of a D&C Red No. 35 Lake (made by extending the dye on insoluble substrata) were analyzed spectrophotometrically. Weighed samples were refluxed with chloroform to dissolve the color. With lakes, filtering the solution

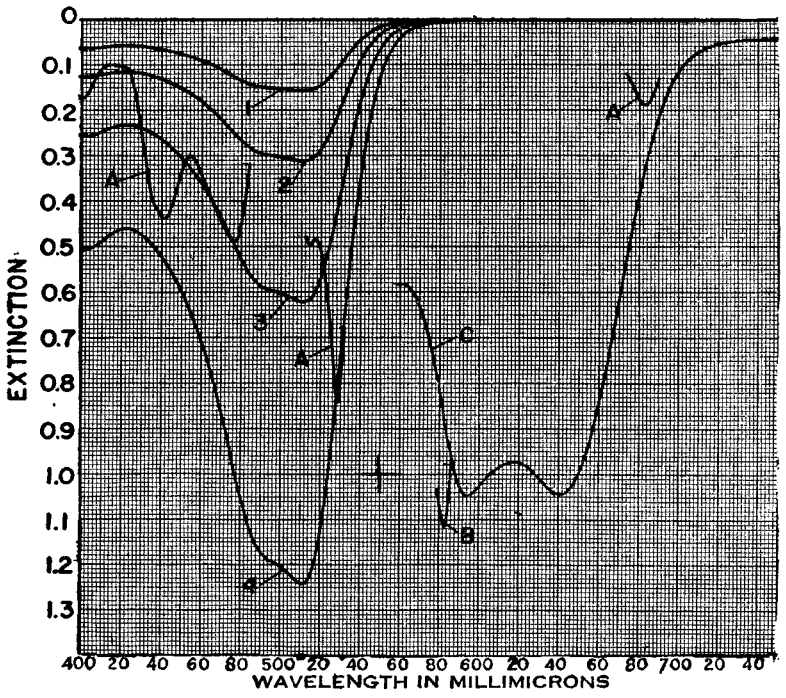

CHART 1.—Pure D&C Red No. 35 in Chloroform.

Curve 1—2.06 mg./liter.
Curve 2—4.13 mg./liter.
Curve 3—8.26 mg./liter.
Curve 4—16.52 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.

through a fine sintered glass crucible to remove the substratum was necessary. The solutions were brought to room temperature, transferred to 100 ml flasks, and made to volume. Extinction measurements were made on suitably diluted aliquots. The curves are shown on Chart 2, and the data presented in Table 2.

D&C RED NO. 36

Experimental

Preparation of Standard Dyestuff.—2-chloro-4-nitroaniline (m.p. 106°C.) was diazotized and coupled with β-naphthol (m.p. 116–117°C.—on

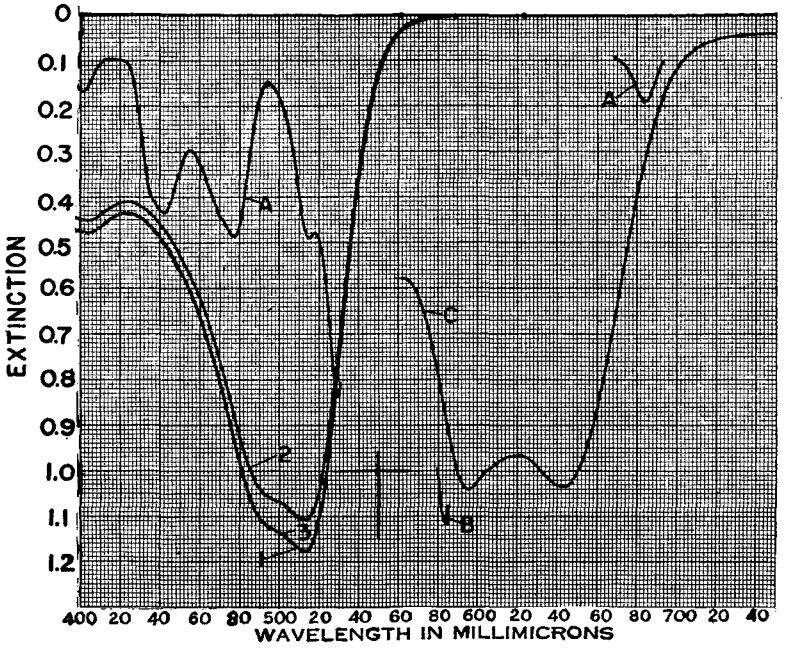

CHART 2.—Certified Samples D&C Red No. 35 in Chloroform.

Curve 1—Sample 1—16.10 mg./liter.
Curve 2—Sample 2—15.33 mg./liter.
Curve 3—Sample 3—16.81 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 Red No. 35*

SAMPLE NO.	M.P.	CONCENTRATION OF SAMPLE	$E_{511\ m\mu}$	DYE* SPECTROPHOTOMETRICALLY	DYE FROM NITROGEN CONTENT
	°C.	*mg./liter*		*Per cent*	*Per cent*
1—Prim. Color	270	16.10	1.178	97.0	97.2
2—Prim. Color	269	15.33	1.106	95.7	95
3—Lake†		16.81	1.174	92.6	90

* The dye content was calculated by using .0754 (Table 1) as the extinction value for 1 mg./liter of pure D&C Red No. 35.
† Substratum—Blanc Fixe.

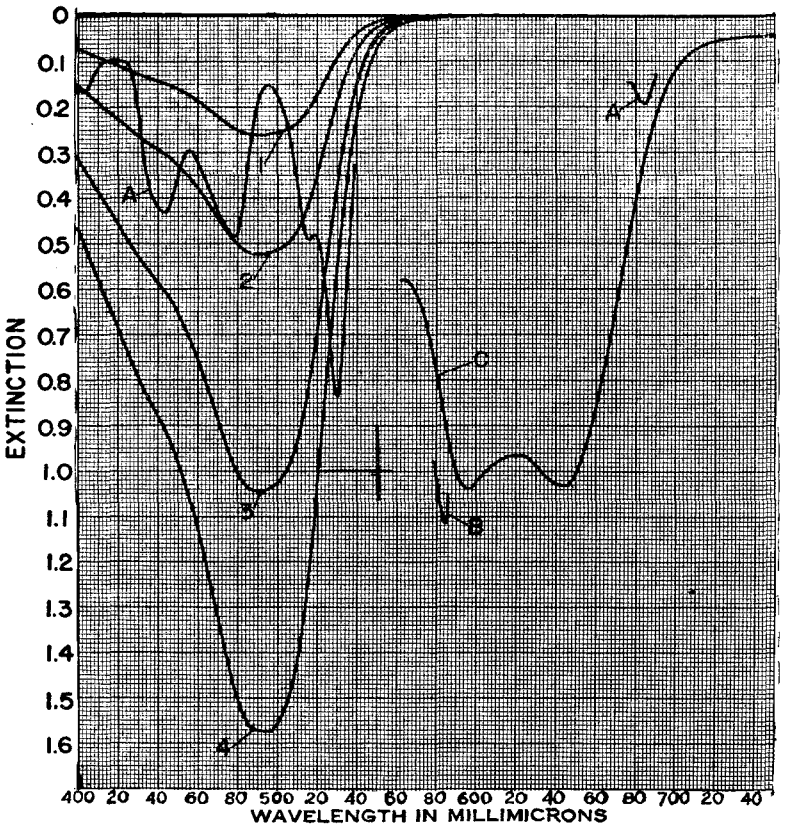

CHART 3.—Pure D&C Red No. 36 in Chloroform.

Curve 1—3.13 mg./liter.
Curve 2—6.26 mg./liter.
Curve 3—12.52 mg./liter.
Curve 4—18.78 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.

Fisher block) in alkaline solution. The product was recrystallized twice from chloroform; it melted at 287–288°C. (literature[5] m.p. 279°C.).

Organically combined chlorine was determined on a portion of the

[5] Beilstein's Handbuch der Organischen Chemie, 4th Edition, Vol. XVI, p. 165 (System No. 2120).

purified material and gave a figure very close to the theoretical value. Theoretical 10.82 per cent; found 10.84 per cent.

Preparation of Solutions.—A 12.52 mg portion of dye was weighed and dissolved in 100 ml of U.S.P. chloroform. Aliquot portions were diluted with chloroform to the concentrations shown in Table 3. Chloroform solutions of D&C Red No. 36 were found to be stable over a period of 4 hours.

TABLE 3.—*Extinction values of solutions of D&C Red No. 36 in U.S.P. chloroform*

Typical Data

CURVE NO. (CHART 3)	CONCENTRATION MG/LITER	EXTINCTION			$E_{490\ m\mu}$ CONCENTRATION	$\frac{E_{470\ m\mu}}{E_{510\ m\mu}}$
		470 mμ	490 mμ	510 mμ		
1	3.13	.226	.262	.232	.0837	.974
2	6.26	.452	.524	.464	.0837	.974
3	12.52	.904	1.046	.926	.0835	.976
4	18.78	1.358	1.576	1.392	.0839	.976
					av. .0837	.975

Spectrophotometric Data.—The extinction curves for the chloroform solutions of D&C Red No. 36 are shown in Chart 3. They show an absorption peak at 490 ± 2 mμ. Wave lengths were corrected as before; see footnote to charts.

The ratio of extinction values at 470 and 510 mμ was calculated. This ratio $E_{470\ m\mu}/E_{510\ m\mu} = .98 \pm .01$. See Table 3.

DISCUSSION

The ratios of extinction to concentration in Table 3 show that at 490 mμ Beer's law is applicable to chloroform solutions of D&C Red No. 36 containing 3.1 to 18.8 mg of color per liter. The pure dye content of a sample of this color can therefore be determined by spectrophotometric examination of its solution in chloroform.

APPLICATION TO COMMERCIAL SAMPLES

Primary Colors and Lakes

The curves shown in Chart 4 and the data in Table 4 were obtained from commercial samples of D&C Red No. 36. Solutions were prepared by the procedure described for D&C Red No. 35.

SUMMARY

Spectrophotometric data for chloroform solutions of purified D&C Red No. 35 and D&C Red No. 36 are presented. Beer's law is shown to be applicable to both colors. The absorption peak for D&C Red No. 35 is at

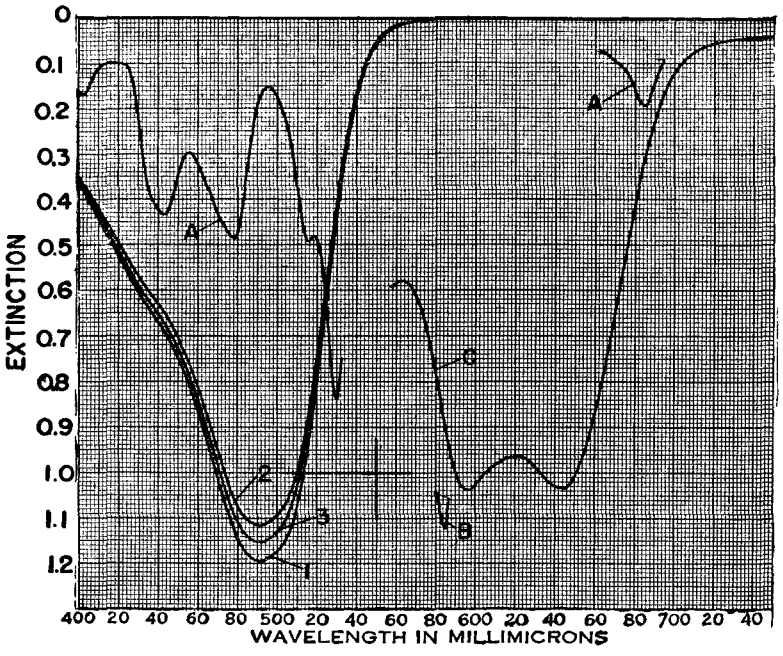

CHART 4.—Certified Samples D&C Red No. 36 in Chloroform.

Curve 1—Sample 1—14.43 mg./liter.
Curve 2—Sample 2—14.43 mg./liter.
Curve 3—Sample 3—14.98 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 4.—*Analysis of certified samples of D&C Red No. 36*

SAMPLE NO.	M.P.	CONCENTRATION OF SAMPLE	E_{430} mμ	DYE* SPECTROPHOTOMETRICALLY	DYE FROM CHLORINE CONTENT
	°C	*mg./liter*		*Per cent*	*Per cent*
1.—Prim. Color	283–4	14.43	1.194	98.8	98.9
2—Lake†		14.43	1.116	92.3	94.4
3—Lake†		14.98	1.154	91.9	94.1

* The dye content was calculated by using .0838 as the extinction value for 1 mg/liter of pure D&C Red No. 36, as obtained from several sets of data similar to Table 3.
† Substratum—Blanc Fixe.

511 mμ; extinction per milligram/liter is .0754 at this wave length, and the extinction ratio E_{500} mμ/E_{520} mμ is 1.06 ± .01.

The absorption peak for D&C Red No. 36 is at 490 mμ; extinction per milligram/liter is .0838 at this wave length, and the extinction ratio E_{470} mμ/E_{510} mμ is .98 ± .01.

Application is made of these data to the determination of pure dye in commercial samples of the colors. Typical results are given.

No report was made on subsidiary dyes in D&C colors.

REPORT ON PHOSPHORIC ACID: NEUTRAL AMMONIUM CITRATE AND TWO PER CENT CITRIC ACID SOLUTIONS AS SOLVENTS FOR ALPHA PHOSPHATE*

By K. D. JACOB, *Associate Referee*, F. N. WARD, W. L. HILL, and CECIL PINKERTON[1] (Division of Soils, Fertilizers, and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland)

Some years ago the availability of calcined phosphate, one type of defluorinated phosphate rock, was investigated in the laboratory and greenhouse by W. H. Ross,[2] the Associate Referee on Phosphoric Acid, and the results were reported at the 1936 meeting of this Association (28), with the recommendation that further study be made of the subject, especially as regards the nature of the citrate-insoluble constituents of the material. At about the same time other members of the staff of this laboratory studied (14) some of the analytical factors—fineness and weight of sample; *p*H of solvent; agitation, temperature, and duration of digestion; water extraction prior to and presence of paper pulp during digestion—that influence the "solubility" of defluorinated phosphate in neutral ammonium citrate solution. It was also shown that calcined phosphate is somewhat more soluble in 2% citric acid than in neutral ammonium citrate. Recently other workers (18) studied certain factors that influence the "solubility" of fused tricalcium phosphate in 2% citric acid solution.

At the preceding meeting of this Association (October 25, 26, 1944), the Referee on Fertilizers recommended (7) that the Associate Referee on Phosphoric Acid study, among other things, methods for the determination of available P_2O_5 in fused tricalcium phosphate, calcium metaphosphate, and potassium metaphosphate. As one phase of such a study, an

* Presented at the annual meeting of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.

[1] Present address, Portland Cement Association Fellowship at National Bureau of Standards, Washington 25, D. C.

[2] Deceased.

investigation was made of some of the factors influencing the "solubility" of fused tricalcium phosphate and other types of defluorinated phosphate rock in neutral ammonium citrate and 2 per cent citric acid solutions, with special reference to the relation between the "solubility" of the materials and their chemical and physical compositions. The present report is based on new work and on previously published data.

DEFINITION OF TERMS

The term *alpha phosphate* is used to denote the products obtained by heating natural 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 (5, 12, 19, 26). It does not include either 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—sintered, fused, and calcined—all of which are frequently called "defluorinated phosphate rock" or "defluorinated phosphate."

Fused alpha phosphate has been manufactured on a large scale by the Tennessee Valley Authority since June, 1945, by defluorinating a melt of silica-bearing phosphate rock at 1400 to 1480°C (tapped melt) in oil-fired shaft-type furnaces and quenching the defluorinated melt in a violent spray of water (5, 6, 9). The product contains about 25 to 30 per cent of P_2O_5, 20 to 25 per cent of SiO_2, 7 to 9 per cent of R_2O_3, and 0.2 to 0.4 per cent of fluorine. While it is currently called "fused tricalcium phosphate" (9, 18, 19, 30), it has also been designated as "fused rock phosphate" (17), "fused phosphate rock" (11, 16, 23, 28), and "fused phosphate."

Sintered alpha phosphate has been produced commercially since June, 1944, by defluorinating mixtures of phosphate rock, or fluorapatite, and silica in rotary kilns at 1550 to 1600°C. (charge temperature) and allowing the product to cool in air. The material contains about 20 per cent of P_2O_5, 45 per cent of SiO_2, 1 to 2 per cent of R_2O_3, and 0.05 to 0.15 per cent of fluorine. While it is marketed under the name of "Coronet defluorinated phosphate," it has also been called "calcined phosphate" (11, 23).

Calcined alpha phosphate has been made experimentally by defluorinating silica-bearing phosphate rock, or fluorapatite, at 1400°C. or higher and allowing the product to cool in air (15, 21, 26). These experimental products contained 34 to 39 per cent of P_2O_5, 5 to 12 per cent of SiO_2, 2 to 4 per cent of R_2O_3, and 0.01 to 0.1 per cent of fluorine. It has not been produced on a commercial scale. It has been generally called "calcined phosphate."

COMPOSITION OF ALPHA PHOSPHATES

Alpha tricalcium phosphate is the principal crystalline phosphate constituent of all three types of alpha phosphate (5, 6, 9, 10, 11, 19) when

the fluorine content is 0.5 per cent or less and appreciable reversion of the phosphorus to other forms has not occurred during the cooling of the product (15, 21, 22, 25, 27). Apatite (in the generic sense) is a major crystalline phosphate constituent only in partially defluorinated products or in material that has reverted during cooling, though identifiable amounts of it are present in most samples. The materials also contain more or less glass (9, 10, 11) (Table 1) which has heretofore been lightly considered as calcium silicate glass and otherwise disregarded. Nevertheless, this glass is unquestionably phosphate-bearing and wherever the proportion of glass is relatively high it must also be regarded as a principal phosphate constituent. Silica (cristobalite) is a major constituent of high-silica alpha phosphate with a low glass content (11) and appears frequently as a minor constituent (often mere traces) in the low-silica products. Other observed minor phosphate constituents are: silicocarnotite and a crystal (phase B) of unknown composition in calcined alpha phosphate (10), and *beta* tricalcium phosphate in fused alpha phosphate (9).

Sintered alpha phosphate is characterized by the presence of a relatively large amount (30 to 40%) of cristobalite and a moderate amount of glass, whereas the fused product is characterized by the presence of minor amounts of cristobalite and much glass (up to 50%) with inbedded crystals of *alpha* tricalcium phosphate. Calcined alpha phosphate is characterized by the absence of more than traces of cristobalite and the presence of considerable glass (up to 30%).

Special interest attaches to three of the phosphate constituents of alpha phosphate—*alpha* tricalcium phosphate, apatite, and glass—not only because of their importance from the viewpoint of the quantity that may be present, but also because of the variable composition of the constituents themselves. *Alpha* tricalcium phosphate takes calcium orthosilicate into solid solution to a marked extent (2, 10) and the amounts, as well as proportions, of calcium oxide and silica in the tricalcium phosphate decrease with increase in the SiO_2 to CaO ratio in the furnace charge. The presence of silicate in solid solution increases the indices of refraction of the tricalcium phosphate and influences its thermal reversion tendencies without markedly affecting its solubility (10).

The apatite found in alpha phosphate is either residual incompletely defluorinated rock or material that crystallized in the mixture during thermal treatment. The variety of apatite no doubt depends upon its origin, but identification of varieties is not possible by x-ray powder diffraction methods and can be accomplished only under especially favorable circumstances by optical methods. The relationship between citrate-solubility of the phosphorus and the residual fluorine content in laboratory preparations of calcined alpha phosphate of low glass content points definitely to the presence in this type of material of a hydroxyl-fluorapatite (containing equivalent amounts of F and OH) (21, 25, 26, 27) that is

TABLE 1.—*Morphology of alpha phosphates*[a]

SAMPLE NO.	FLUORINE	GLASS[b]	CRYSTAL COMPOSITION[c]	GENERAL APPEARANCE[c]
	per cent	*per cent*		
			Sintered alpha phosphate	
2362	1.02	60	Minor C, minor quartz (large), rare A	Tiny C and large A imbedded in G ($n > 1.60$)
2377	0.43	40	Abundant C (fine, scaly), some quartz(?), minor A	Crystals cemented together with G ($n < 1.55$); some G with high index
2340	0.10	20	Abundant crystals with high index cemented with G; still more abundant cristobalite and G with low index	Very fine-grained
2490	0.05	30	Abundant C (thin flakes), trace A	Fine crystal-like droplets, apparently G, with very low index in masses among C crystals
			Fused alpha phosphate	
2405	1.11	65	Feathery masses of needles, high index and low biref.' presumably A	Crystals imbedded in G; some clear G, some free crystals
2401	0.47	30	Abundant C (large, well-formed), some A	Crystals cemented together with G; G fragments and colored G
2496	0.34	20	Abundant C, some A	Feathery masses of C; well-formed A; fragments of clear G with low index
2394	0.02	25	Abundant C, trace A	Crystals mixed with G of low index
			Calcined alpha phosphate	
8	1.49	60	Two materials: (a) green high-index G as network around medium ovoid C; (b) paler G with apparently lower index enclosing needle-like masses of crystallites	
1351	0.68	20	Abundant C, trace A (fairly large)	Green interstitial G of high index
1326	0.31	30	Abundant C with enhanced indices (1.597) trace A	Yellow interstitial G; clean platy prismatic crystals
1374	0.15	15	Abundant C with enhanced indices, trace A	Ovoid C imbedded in yellowish G; some clear G

[a] Optical examination of the materials was made by J. G. Cady of this Bureau.
[b] Percentage of glass should be regarded as a rough estimate.
[c] Apatite, *alpha* tricalcium phosphate and glass, respectively, are designated by the letters A, C, and G.

presumed to be partially defluorinated apatite from the rock. Fully fluorinated fluorapatite in coarse crystals has been identified by optical methods in air-cooled samples of fused alpha phosphate (9). Since this apatite probably crystallized during the cooling of the melt, it is presumed to be the form that occurs in the water-quenched product (9). Other apatites that apparently involve neither fluorine nor hydroxyl have been observed in reverted synthetic preparations of calcined alpha phosphate (10).

The glass in alpha phosphate contains the calcium oxide and phosphoric oxide not in the crystalline phosphate constituents, all of the aluminum and iron oxides, considerable silica and probably a little fluorine, besides a rather long list of minor constituents of the phosphate rock. The actual composition of the glass and its amount in a particular case depend upon the heat treatment and quenching technique, as well as upon the composition of the furnace feed. Although the composition of the glass constituent has not been studied formally, some idea of its probable composition is obtainable from sundry considerations. Glasses containing as much as 15 per cent of P_2O_5 (45 to 50% CaO and 30 to 40% SiO_2) can be prepared without difficulty;[3] and rapid quenching of a melt would doubtless increase appreciably the amount and P_2O_5 content of the glass. Accordingly, one can with good reason expect at least 3 to 6 per cent of P_2O_5 to reside in the glass in alpha phosphates that contain 20 to 40 per cent of glass. That the glass also carries some of the fluorine seems plausible, since a commercial phosphate-bearing glass (20% P_2O_5) of relative high fluorine content is now being produced by fusing phosphate rock with magnesium silicate. Finally, it should be pointed out that the presence of more than traces of cristobalite in an alpha phosphate indicates that the glass is saturated with silica.

MATERIALS

Pure Compounds.—Most of the pure compounds—hydroxylapatite, silicocarnotite, and the *alpha* and *beta* forms of tricalcium phosphate—used in this study were prepared for previous investigations. Their sources and methods of preparation are indicated in the appropriate tables.

Natural Phosphates.—The phosphate rocks and apatites are from the Bureau's collection of samples of commercial materials accumulated during the past 20 years.

Alpha Phosphates.—The samples of sintered alpha phosphate are from products manufactured on a commercial scale.[4] Nos. 2296, 2350 to 2365, and 2490 were made from Florida land-pebble phosphate. No. 2378 was prepared from apatite concentrate produced in the Kola Peninsula, U.S.S.R. and the other samples were made from apatite mined at Piney River, Va.

[3] Unpublished data of this Bureau.

[4] The samples were supplied by the Coronet Phosphate Company, West Conshohocken, Pa. This operation has now been transferred to Plant City, Fla.

TABLE 2.—*Effect of weight of sample on "solubility" in neutral ammonium citrate of alpha phosphates and related materials*

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL	TOTAL P_2O_5	F	WEIGHT OF SAMPLE PER 100 ML. OF EXTRACTANT	FRACTION OF TOTAL P_2O_5 EXTRACTED		
					BY NEUTRAL AMMONIUM CITRATE	BY 2% CITRIC ACID	DIFFERENCE
		per cent	*per cent*	*gram*	*per cent*	*per cent*	*per cent*
2296[a,b]	Sintered alpha phosphate	20.7	0.02	1.0	95.0	95.2	0.2
				0.5	95.0	96.4	1.4
2224[a,b]	Fused alpha phosphate	28.5	0.02	1.0	85.5	89.2	3.7
				0.5	94.1	93.3	−0.8
1351[c,d]	Calcined alpha phosphate	36.58	0.68	1.0	67.7	70.1	2.4
				0.75	68.2	70.0	1.8
				0.5	68.4	70.4	2.0
				0.25	68.6	72.5	3.9
1374[c,d]	Calcined alpha phosphate	35.17	0.15	1.0	88.9	91.8	2.9
				0.75	90.0	91.9	1.9
				0.5	90.1	91.5	1.4
				0.25	90.4	92.7	2.3
2262[b,e]	Tricalcium phosphate, *alpha* form	46.3	0.00	1.0	89.4	92.3	2.9
				0.5	91.5	95.2	3.7
2255[b,e]	Florida waste-pond phosphate[f]	22.2	1.78	1.0	12.5	30.1	17.6
				0.5	19.8	45.5	25.7
1934[b,e]	Florida land-pebble phosphate	31.3	3.75	1.0	11.6	20.1	8.5
				0.5	16.4	33.4	17.0

[a] Ground to pass the 80-mesh sieve (177-micron opening).
[b] Results given by Hill *et al* (11).
[c] Ground to pass the 80-mesh sieve (175 micron opening).
[d] Results given by Jacob, Rader, and Tremearne (14).
[e] Ground to pass the 100-mesh sieve (149-micron opening).
f Commonly called "colloidal phosphate," and designated by the Association of Official Agricultural Chemists as "soft phosphate with colloidal clay" (1, p. 901).

The samples of fused alpha phosphate are mainly pilot-plant materials made by the Tennessee Valley Authority. Nos. 2394 to 2398 and 2399 to 2401 were prepared, respectively, from Tennessee brown-rock and Florida land-pebble phosphates on the pilot-plant scale in an oil-fired, shaft-type furnace, whereas No. 2402 and Nos. 2403 to 2405 were made, respectively, from the Tennessee and Florida phosphate on the pilot-plant scale by fusion in a single-electrode arc furnace and subsequent defluorination in an oil-fired, hearth-type furnace. Sample 2496 was produced on a commercial scale at Godwin, near Columbia, Tennessee (9).

In the case of the calcined alpha phosphates, Nos. 6, 11, and 1478 and 1322 to 1374 are pilot-plant materials made from Tennessee brown-rock phosphate in oil-fired rotary kilns.[5] The other samples are laboratory materials prepared in this Bureau.

METHODS OF ANALYSIS

Extraction Procedures.—All the neutral ammonium citrate extractions reported herein were made by the official method for non-acidulated samples other than basic slag (1, pp. 24–25). Accordingly, the citrate digestions were made at 65°C in the presence of filter paper with shaking at 5-minute intervals. In the case of the natural phosphates and the unignited hydroxylapatites, the citrate extracts were filtered through Pasteur-Chamberland tubes in order to obtain clear filtrates. One-gram samples of the phosphates were used except in the experiments designed to show the effect of weight of sample on the quantity of extracted P_2O_5 (Table 2).

The citric acid extractions were made according to the official method (1, p. 25). Except for the experiments with different sample weights (Table 2), the extractions were in the ratio of 1 gram of sample per 100 ml. of acid.

Effect of Weight of Sample.—The effect of variations in sample weight per unit volume of solvent, on the quantity of P_2O_5 extracted from alpha phosphates and related materials by neutral ammonium citrate and by 2 per cent citric acid, has been investigated briefly by Hill *et al.* (11) and by Jacob, Rader, and Tremearne (14), whose results are summarized in Table 2. The results for sintered and calcined alpha phosphates were only slightly affected by decreasing the sample weight to 0.25–0.5 gram. It therefore appears that practically complete extraction of the readily soluble P_2O_5 in these two types of alpha phosphate can be effected by the use of 100 ml. of reagent per gram of material. On the other hand, the effect of sample weight was appreciable in the case of *alpha* tricalcium phosphate and fused alpha phosphate and was especially marked in the case of the mineral phosphates.

Effect of Particle Size.—The influence of fineness of sample on the

[5] The samples were supplied in 1933 and 1935 by the Moorman Manufacturing Company, Quincy, Ill., and Darling and Company, East St. Louis, Ill.

"solubility" of calcined and fused alpha phosphates has been studied by several workers (14, 16, 18, 28). New results obtained on sintered and fused materials and on Florida land-pebble phosphate are presented in Figure 1, where published results for calcined phosphate (14) are repro-

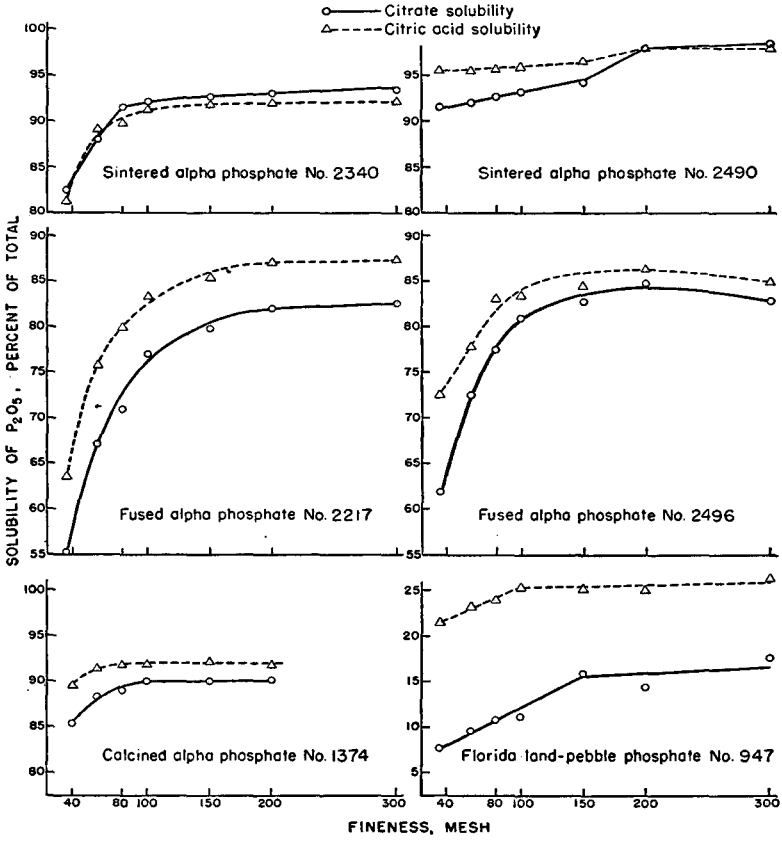

FIG. 1.—Effect of Fineness of Sample on the "Solubility" of Alpha Phosphate and Phosphate Rock

duced for purposes of comparison. The mechanical composition of these materials determined by dry-sieving is given in Table 3.

The solubility-fineness data (Fig. 1) show the fineness to which the different types of alpha phosphates should be ground in order to reduce the particle-size effect on the solubility to negligible values. A rigid interpretation of the results points to a fineness of −100 mesh for calcined material and −200 mesh for sintered and fused materials. However, a fine-

TABLE 3.—*Mechanical composition of alpha phosphates ground to different finenesses*

SAMPLE NO.	FINENESS[a]	COMPOSITION, MESH						
		−35, +60	−60, +80	−80, +100	−100, +150	−150, +200	−200, +300	−300
	mesh	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Sintered Materials								
2340	− 35	39.0	14.5	10.2	9.6	10.8	4.0	11.9
	− 60	—	19.9	19.5	18.3	17.8	7.1	17.4
	− 80	—	—	8.7	20.4	30.1	12.4	28.4
	−100	—	—	—	15.3	35.9	14.9	33.9
	−150	—	—	—	—	37.6	19.8	42.6
	−200	—	—	—	—	—	25.2	74.8
2490	− 35	5.2	9.2	12.2	15.6	20.8	11.0	26.0
	− 60	—	7.2	10.8	16.4	22.0	11.8	31.8
	− 80	—	—	7.6	17.4	23.6	16.0	35.4
	−100	—	—	—	14.2	24.2	18.8	42.8
	−150	—	—	—	—	26.0	23.0	51.0
	−200	—	—	—	—	—	18.6	81.4
Fused Materials								
2217	− 35	42.5	23.2	13.6	8.3	5.2	2.4	4.8
	− 60	—	19.0	23.9	19.4	15.1	6.7	15.9
	− 80	—	—	16.0	24.4	21.3	10.3	28.0
	−100	—	—	—	17.5	26.2	15.0	41.3
	−150	—	—	—	—	30.3	19.2	50.5
	−200	—	—	—	—	—	23.4	76.6
2496	− 35	45.0	20.4	10.4	8.4	6.2	3.4	6.2
	− 60	—	20.4	17.6	17.6	16.4	8.0	20.0
	− 80	—	—	11.0	22.6	25.6	12.4	28.4
	−100	—	—	—	19.6	30.0	14.6	35.8
	−150	—	—	—	—	29.8	18.8	51.4
	−200	—	—	—	—	—	19.6	80.4
Calcined Material[b]								
1374	− 40	11.4[c]	8.6	8.9	14.3	14.8	42.0	—
	− 60	—	7.4	12.6	16.9	17.1	46.0	—
	− 80	—	—	8.3	14.2	19.2	58.3	—
	−100	—	—	—	9.6	18.3	72.1	—
	−150	—	—	—	—	29.5	70.5	—
Florida Land-pebble Phosphate								
947	− 35	27.8	12.2	7.8	9.2	7.2	16.6	19.2
	− 60	—	11.6	7.2	8.8	13.2	25.4	33.8
	− 80	—	—	5.2	9.8	18.0	33.8	33.2
	−100	—	—	—	8.0	10.0	38.6	43.4
	−150	—	—	—	—	8.4	47.0	44.6
	−200	—	—	—	—	—	27.8	72.2

[a] Screen openings in sieve series were 420, 250, 177, 149, 104, 74, and 46 microns, respectively, except in instances where other dimensions are indicated.
[b] Results given by Jacob, Rader, and Tremearne (14). Screen openings were 381, 221, 175, 147 and 104 microns, respectively.
[c] Mesh size was −40, +60.

ness of −100 mesh for the latter two types of material would sacrifice only 2 to 4 per cent of the phosphorus to the insoluble fraction.

The peculiar shape of the citrate solubility curve for one of the sintered materials (No. 2490) is probably caused by the odd particle-size distribution in comparison with the other materials (Table 3). This consideration raises the question of reproducibility of particle-size distributions, which may have an important bearing on analytical variability when the fineness of the sample does not lie in the range of a nearly horizontal portion of the solubility-fineness curve. The other curves (Fig. 1) have the same form. The citric acid solubility is usually, but not always, higher than the citrate solubility, and the difference has a substantially constant value for the fine and moderately fine grinds.

SOLUBILITY OF PHOSPHATES

Pure Materials.—The "solubilities" of several preparations of hydroxylapatite, *beta* and *alpha* tricalcium phosphates, and silicocarnotite are given in Table 4. On the basis of citrate solubility the materials may be divided into two groups—one, consisting of hydroxylapatite and *beta* tricalcium phosphate, with "solubilities" of about 50 per cent or less, the other comprising *alpha* tricalcium phosphate and silicocarnotite, with "solubilities" above 70 per cent. Citric acid solubilities are higher in every case and markedly so for materials in the group with medium to low citrate solubility. Consequently, citric acid solubility does not show as wide a difference between materials such as hydroxylapatite and silicocarnotite as does citrate solubility. On the other hand, silicocarnotite was found to be an excellent source of phosphorus for wheat and Sudan grass in a series of greenhouse experiments on soils of *p*H 5.7 to 5.9 (4, 29), in which hydroxylapatite gave results that were little better than those obtained with phosphate rock. Reliable preparations of the tricalcium phosphates have not, as far as the authors know, been tested in plant response experiments.

The preparations of tricalcium phosphate show considerable variation is "solubility." The *alpha* form, being a major phosphate constituent of alpha phosphate, has special interest in this connection. The results, ranging in percentages from the lower seventies to the upper nineties, emphasize both the difficulty involved in the preparation of reliable samples and the need for great care in the choice of material for experiment.

Natural Phosphates.—The "solubilities" of typical fluorapatites and phosphate rocks used in the fertilizer industry are given in Table 5.[6] The different types of materials, which are essentially varieties of apatite (3, 8, 13, 20, 24), are listed in the order of increasing solubility in neutral ammonium citrate solution. The citrate solubilities range from about 2 per cent for fluorapatite to 34 per cent for phosphate from Denis Island.

[6] The analyses were made by T. H. Tremearne, formerly of this Bureau, and D. S. Reynolds, deceased.

TABLE 4.—*"Solubility" of some pure compounds related to alpha phosphate*

SAMPLE NO.	SOURCE OR TYPE OF MATERIAL	TOTAL P_2O_5	FRACTION OF TOTAL P_2O_5 EXTRACTED		
			BY NEUTRAL AMMONIUM CITRATE	BY 2% CITRIC ACID	DIFFERENCE
		per cent	*per cent*	*per cent*	*per cent*
	Hydroxylapatite				
2187[a,b]	Precipitated material, air-dried	41.4	24.3	58.1	33.8
2187[a,b]	Precipitated material, heated at 900°C.	42.1	21.8	58.1	36.3
	***Beta* Tricalcium Phosphate**				
2466-a[c]	Precipitated tricalcium phosphate heated at 900°C.	45.3	53.5	89.0	35.5
2500[a]	Precipitated tricalcium phosphate heated at 1050°C.	45.2	48.2	90.6	42.4
2466-c[c]	Crystallized from *alpha* form at 1000°C.	45.5	41.3	78.0	36.7
2262-b[a,b]	Crystallized from *alpha* form at 1000°C.	46.3	39.5	64.9	25.4
2268[a,b]	Crystallized from *alpha* form at 1000°C.	44.9[d]	41.9	76.0	35.1
	***Alpha* Tricalcium Phosphate**				
2466-b[e]	Precipitated tricalcium phosphate heated at 1400°C.	45.5	95.7	97.1	1.4
2262-a[a,b]	Precipitated tricalcium phosphate heated at 1400°C.	46.3	89.4[e]	92.3[e]	2.9
2267[a,b]	Precipitated tricalcium phosphate heated at 1400°C.	45.1[f]	71.0	75.0	4.0
	Silicocarnotite				
2188-b[a,b]	Prepared by heating mixture of precipitated tricalcium phosphate, cristobalite and calcium carbonate at 1600°C.	29.0	95.9	100.0	4.1

[a] Ground to pass the 100-mesh sieve (149-micron opening).
[b] Results given by Hill *et al* (11).
[c] Ground to pass the 150-mesh sieve (104-micron opening).
[d] Contains 0.13% of fluorine.
[e] The same result was obtained on material ground to pass the 150-mesh sieve.
[f] Contains 0.02% of fluorine and some *beta* tricalcium phosphate.

TABLE 5.—*"Solubility" of natural calcium phosphates*

SAMPLE NO.[a]	TYPE OR SOURCE OF PHOSPHATE	TOTAL P_2O_5	F	P_2O_5:F RATIO	FRACTION OF TOTAL P_2O_5 EXTRACTED		
					BY NEUTRAL AMMONIUM CITRATE	BY 2% CITRIC ACID	DIFFERENCE
		per cent	*per cent*		*per cent*	*per cent*	*per cent*
	Fluorapatite						
1291	U.S.S.R.: Kola Peninsula	39.30	3.08	12.8	1.7	6.0	4.3
1295	Virginia: Nelson County	39.50	2.83	14.0	2.1	8.4	6.3
	Phosphate Rock from the United States						
1407	Montana: Avon	37.94	3.96	9.6	4.8	14.2	9.8
1009	Montana: Garrison	31.39	3.21	9.8	5.2	15.5	10.3
571	Tennessee blue rock	27.90	3.27	8.5	5.1	21.6	16.5
576	Tennessee blue rock	31.22	3.58	8.7	5.8	20.8	15.0
774	Tennessee brown rock	35.01	3.80	9.2	5.3	17.4	12.1
587	Tennessee brown rock	30.17	3.18	9.5	5.9	19.4	13.5
762	Tennessee brown rock	33.73	3.76	9.0	6.9	17.4	10.5
1411	Idaho: Georgetown	30.30	3.19	9.5	6.9	17.1	10.2
1412	Idaho: Paris	35.39	3.76	9.4	8.0	23.7	15.7
932	Florida hard rock	35.99	3.89	9.3	8.0	20.1	12.1
589	Florida hard rock	34.68	3.73	9.3	8.2	18.5	10.3
1270	Florida land pebble	36.47	3.89	9.4	7.8	20.7	12.9
622	Florida land pebble	33.56	3.92	8.6	9.9	19.0	9.1
947	Florida land pebble	31.28	3.89	8.0	11.2	25.3	14.1
618	Florida land pebble	30.53	3.79	8.1	12.7	21.4	8.7
725	Florida waste pond[b]	25.31	2.20	11.5	9.7	27.2	17.5
828	Florida waste pond[b]	19.83	2.01	9.9	11.1	40.3	29.2
	Phosphate Rock from Africa						
1593	Egypt	30.00	3.67	8.2	13.5	38.5	25.0
1162	Morocco	35.11	4.24	8.3	14.1	32.0	17.9
1571	Morocco	31.86	4.15	7.7	15.0	35.0	20.1
552	Tunisia	27.55	3.46	8.0	15.9	36.6	20.7
1557	Algeria	28.17	3.55	7.9	16.9	36.7	19.8
	Insular Phosphate Rock						
451	Ocean Island	40.32	3.08	13.1	7.7	21.7	14.0
1600	Angaur Island	38.92	2.94	13.2	9.1	25.7	16.6
1567	Nauru Island	39.25	2.58	15.2	9.6	26.6	17.0
1159	Makatea Island	38.22	3.20	11.9	11.1	24.0	12.9
452	Christmas Island	39.46	1.51	26.1	12.0	32.4	20.4
1231	Christmas Island	40.03	1.27	31.5	13.2	32.6	19.4
1546	Curacao Island	35.39	0.96	36.9	11.0	30.6	19.6
1544	Curacao Island	39.48	0.75	52.6	14.0	36.1	22.1
1297	Assumption Island[c]	30.40	0.57	53.3	31.1	64.0	32.9
1296	Denis Island[c]	32.88	0.48	68.5	34.2	62.3	28.1

[a] The samples were ground to pass the 100-mesh sieve. The screen opening was 147 microns except in the case of sample 947 (149 microns). Results are on the moisture-free basis (105°C.).

[b] Commonly called "colloidal phosphate," and designated by the Association of Official Agricultural Chemists as "soft phosphate with colloidal clay" (1, p. 901).

[c] Seychelles group; powdery material of the phospho-guano type.

Domestic rocks stand next to fluorapatite in this series, and the insular rocks show the widest variation in "solubility." The results for citric acid solubility, which are two- to four-fold higher, fall approximately in the same order. In the light of known varietal differences among apatites the lack of a consistent general relation between solubility variation and variation in fluorine content is to be expected. The insular phosphates of lowest fluorine content (less than 1 per cent), which may be regarded as fluorinated hydroxylapatite, show high "solubilities" of the order of pure hydroxylapatite (Table 4).

Alpha Phosphates.—The "solubility" of alpha phosphate is associated historically and functionally with the residual fluorine in the material. In the early laboratory work (21, 25, 26, 27) on the defluorination of phosphate rock the observation was made that solubilization of the phosphorus in calcined alpha phosphate accompanied the removal of the last half of the fluorine equivalent of the phosphorus as $Ca_{10}(PO_4)_6F_2$ and was proportional to the volatilization of fluorine in this range of defluorination. On the basis of this observation it was concluded that a fluor-hydroxylapatite near $Ca_{10}(PO_4)_6FOH$ was the principal intermediate product in the calcination process. Accordingly, partially defluorinated and solubilized products (exclusive of reversion during cooling) were viewed as mixtures of fluor-hydroxylapatite having a low "solubility" and *alpha* tricalcium phosphate with a high "solubility." If it be assumed that the product is essentially a binary mixture of this type, the phosphorus present in the form of *alpha* tricalcium phosphate can be calculated for comparison with the determined "solubility." For purposes of similar calculation and use partially defluorinated fused alpha phosphate has been regarded as a mixture of $Ca_{10}(PO_4)_6F_2$ and α-$Ca_3(PO_4)_2$ (18).

Sufficient "solubility" data (Table 6) are now available to permit a critical examination of the validity of the assumption of binary mixtures for commercial alpha phosphates. In this connection special interest attaches to residual fluorine content ranging from that of fluorapatite (P_2O_5:F = 11.2) to that of fluor-hydroxylapatite (P_2O_5:F = 22.4) and somewhat below as shown in Figure 2.

Considering first the laboratory preparations (26), which are not given in Table 6, it will be noted that the citrate solubility (heavy curve, Fig. 2) at first decreases with increase in the P_2O_5:F ratio (decrease in fluorine content), passes through a minimum and then rises rather rapidly. Although the paucity of data in the critical range and the hyperbolic nature of the curves when the ratio is used as composition variable make the position of the minimum rather indefinite, it is obvious that the upward trend in "solubility" begins near, though on the low side of, the ratio (22.4) for $Ca_{10}(PO_4)_6FOH$. At somewhat higher ratios the "solubilities" tend to fall along the curve (dotted line) for α-$Ca_3(PO_4)_2$ calculated on the assumption that the products are *alpha* tricalcium phosphate and fluor-

TABLE 6.—*"Solubility" of sintered, fused, and calcined alpha phosphates in relation to their fluorine content*

SAMPLE NO.	FINENESS	TOTAL P_2O_5	F	P_2O_5:F RATIO	FRACTION OF TOTAL P_2O_5 EXTRACTED		
					BY NEUTRAL AMMONIUM CITRATE	BY 2% CITRIC ACID	DIFFERENCE
	mesh	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
Sintered alpha phosphate							
2365	150	21.05	1.56	13.7	7.7	21.0	13.3
2364	150	21.25	1.38	15.4	5.3	19.0	13.7
2363	150	21.53	1.24	17.4	14.4	26.4	12.0
2362	150	21.40	1.02	21.0	27.0	36.8	9.8
2377	150	24.04	0.43	55.9	71.3	74.1	2.8
2376	150	23.56	0.34	69.3	77.0	79.6	2.6
2351	150	20.10	0.25	80.4	81.0	84.3	3.3
2357	150	20.30	0.19	106.8	85.3	87.2	1.9
2368	150	23.54	0.20	117.7	83.4	87.3	3.9
2352	150	20.28	0.17	119.3	84.3	87.4	3.1
2370	150	23.15	0.18	128.6	84.1	88.9	4.8
2353	150	20.62	0.15	137.5	86.0	87.4	1.4
2378	150	20.66	0.14	147.6	86.6	90.1	3.5
2371	150	23.05	0.12	192.1	88.1	92.8	4.7
2340	150	20.74	0.10	—	92.6	91.7	−0.9
2350	150	19.88	0.06	—	92.6	96.2	3.6
2490	150	20.42	0.05	—	94.2	96.5	2.3
2296[a]	80	20.70	0.02	—	95.0	95.2	0.2
Fused alpha phosphate							
S-849[b]	100	25.80	1.25	20.6	17.1	26.7	9.6
2405	150	30.01	1.11	27.0	29.8	36.0	6.2
2404	150	30.36	0.92	33.0	39.3	46.8	7.5
2403	150	30.50	0.72	42.4	50.4	56.2	5.8
2401	150	28.83	0.47	61.3	71.7	74.6	2.9
2402	150	30.28	0.37	81.8	63.4	72.0	8.6
2496	150	27.68	0.34	81.4	82.8	84.6	1.8
2398	150	25.75	0.30	85.8	78.9	78.3	−0.6
2400	150	28.58	0.29	98.6	77.3	82.1	4.8
2217	150	29.53	0.28	105.5	79.2	86.0	6.8
2396	150	25.85	0.23	112.4	80.2	82.5	2.3
2399	150	26.10	0.17	153.5	88.5	92.1	3.6
2397	150	29.72	0.15	198.1	87.7	91.4	3.7
2394	150	28.42	0.02	—	95.0	96.9	1.9
2395	150	28.68	0.02	—	90.8	95.4	4.6
Calcined alpha phosphate							
7[c]	100	33.88	2.08	16.3	6.8	16.8	10.0
8[c]	100	34.09	1.49	22.9	20.6	27.7	7.1
9[c]	100	33.38	0.85	39.3	49.1	54.4	5.3
1351	150	36.58	0.68	53.8	68.9	70.7	1.8
1323	150	32.95	0.49	67.2	67.9	74.9	7.0
1324	150	33.49	0.42	79.7	68.9	71.5	2.6
1325	150	33.97	0.40	84.9	66.8	71.3	4.5
1326	150	34.12	0.31	110.1	78.3	81.0	2.7
10[c]	100	33.78	0.27	125.1	78.1	82.6	4.5
1322	150	34.42	0.23	149.7	83.5	85.9	2.4
1374[d]	200	35.17	0.15	—	90.2	91.8	1.6
1327	150	34.21	0.10	—	89.0	92.8	3.8
13[c]	100	35.09	0.10	—	92.7	94.3	1.6
6[e]	80	37.18	0.09	—	90.0	92.5	2.5

[a] Results given by Hill *et al* (11).
[b] Results given by MacIntire and Palmer (18).
[c] Results given by Jacob, *et al* (12).
[d] Results given by Jacob, Rader, and Tremearne (14).
[e] Results given by Jacob and Ross (16).

hydroxylapatite. At the same time the materials prepared on a larger scale by commercial concerns (light curves, Fig. 2) show higher "solubilities" than do the laboratory preparations. Reasonable extension of these curves to lower ratios would appear to strike close to the ratio (11.2) for fluorapatite—a condition that offers some justification for the view that fused

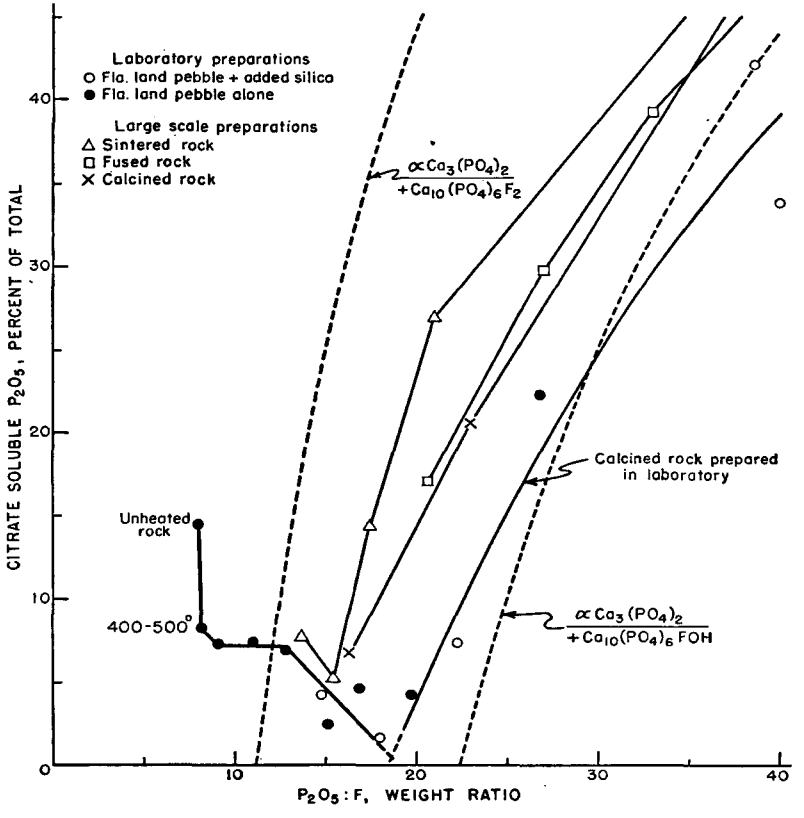

FIG. 2.—Dependence of "Solubility" on the P_2O_5 to F ratio of Alpha Phosphate

alpha phosphate consists of fluorapatite and tricalcium phosphate. On the other hand, examination of these materials with the petrographic microscope reveals the presence of relatively large quantities of glass (Table 1). Furthermore, materials with P_2O_5:F ratios near that of the minimum "solubility" contain, in general, the larger amounts of glass, which is in harmony with the observation (6) that the fusion temperature of partially defluorinated rock is lowest in this range of fluorine content. The laboratory preparations contained only small amounts of glass, although the amounts were not estimated in numerical terms.

Materials with P_2O_5:F ratios of 22.4 or less are, strictly speaking, not alpha phosphates within the bounds of the definition given earlier, because *alpha* tricalcium phosphate is not a major constituent of them. Since the glass constituent doubtless contains phosphate, these materials cannot be properly treated as binary mixtures. Therefore, values for *alpha* tricalcium phosphate calculated from the fluorine content of partially defluorinated products are, in general, practically worthless for comparison with determined "solubilities." To a somewhat less extent the same is true of the more completely defluorinated products.

COMPARISON OF CITRATE AND CITRIC ACID SOLUBILITIES OF ALPHA PHOSPHATES AND RELATED MATERIALS

The citrate solubilities listed in Tables 4, 5, and 6 range from 1.7 to 95 per cent. With the exception of two cases, alpha phosphates No. 2340 and 2398, the citric acid solubility is higher than the citrate solubility, and the latter values range from 6.0 to 100 per cent. Since the results obtained with the use of the two solvents are roughly parallel, it is permissible to use their difference as a basis of comparison. A summary of the data, exclusive of the two exceptional cases, is given in Table 7.

Partially defluorinated rock with P_2O_5 to F ratio of 13.7 to 21.0 (Item 8), though somewhat more soluble than unheated rock (Items 2 and 3), show almost the same differences in "solubilities" by the two methods as does unheated rock, for which the P_2O_5 to F ratios are around 8 to 9. The average difference is 11.4 for partially defluorinated rock in comparison with 11.5 for unheated Florida land pebble and Tennessee brown rock (Item 3). The higher "solubility" of the partially defluorinated materials is attributable to their content of phosphate-bearing glass. On the other hand, alpha phosphates with P_2O_5 to F ratios greater than 22.4 (Item 12), and especially materials defluorinated to less than 0.5 per cent of fluorine (Item 16), show smaller differences (less than 5%) that agree well with those observed on *alpha* tricalcium phosphate and silicocarnotite (Items 6 and 7). Thus, it is seen that the difference between the "solubilities" in the two solvents apparently undergoes an abrupt decrease at about the overall composition represented by the formula $Ca_{10}(PO_4)_6FOH$.

The different types of alpha phosphate show about the same range in "solubility." In the case of materials containing less than 0.5 per cent of fluorine the upper limits of the ranges are well into the nineties (Items 16 to 19), and the lower limits are 63.4, 66.8, and 71.3 per cent, respectively, for fused, calcined, and sintered materials. The order is that of decreasing glass content. The average differences between citric acid and citrate solubilities are in the same order and amount to 4.1, 3.3, and 2.2 per cent, respectively.

SUMMARY

(1) The term *alpha phosphate* is used in this report as a generic name for

TABLE 7.—*Summary of "solubility" data for alpha phosphates and related materials*

ITEM NO.	MATERIAL	SAMPLES INCLUDED IN AVERAGE	RANGE OF CITRATE SOLUBILITY	DIFFERENCE BETWEEN CITRIC ACID AND CITRATE SOLUBILITIES	
				RANGE	AVERAGE
		number	*per cent of total*		
1	Fluorapatite	2	1.7– 2.1	4.3– 6.3	5.3
2	Phosphate rock, U. S. A.	15	4.8–12.7	8.7–16.5	11.9
3	Land pebble and brown rock	7	5.3–12.7	8.7–14.1	11.5
4	Hydroxylapatite	2	21.8–24.3	33.8–36.3	35.1
5	*Beta* tricalcium phosphate	5	39.5–53.5	25.4–42.4	35.0
6	*Alpha* tricalcium phosphate	3	71.0–95.7	1.4– 4.0	2.8
7	Silicocarnotite	1	95.9	—	4.1
8	Partially defluorinated rock, P_2O_5:F <22.4	6	5.3–27.0	9.6–13.7	11.4
9	Sintered material	4	5.3–27.0	9.8–13.7	12.2
10	Fused material	1	17.1	—	9.6
11	Calcined material	1	6.8	—	10.0
12	Alpha phosphates, P_2O_5:F >22.4	39	20.6–95.0	0.2– 8.6	3.7
13	Sintered product	13	71.3–95.0	0.2– 4.8	2.2
14	Fused product	13	29.8–95.0	1.8– 8.6	4.7
15	Calcined product	13	20.6–92.7	1.6– 7.1	3.6
16	Alpha phosphates, F<0.5 per cent	33	63.4–95.0	0.2– 8.6	3.4
17	Sintered product	13	71.3–95.0	0.2– 4.8	2.2
18	Fused product	10	63.4–95.0	1.8– 8.6	4.1
19	Calcined product	10	66.8–92.7	1.6– 7.0	3.3

the sintered, fused, or calcined products obtained by heating natural phosphates at high temperatures in the presence of silica and water vapor for the purpose of converting the P_2O_5 into plant-available forms, principally *alpha* tricalcium phosphate.

(2) The important phosphate-bearing constituents of alpha phosphates are *alpha* tricalcium phosphate, glass, and apatite. The composition of these constituents, especially the latter two, is variable. Crystals of tricalcium phosphate and apatite are often imbedded in the glass.

(3) An investigation was made of neutral ammonium citrate and 2 per cent citric acid solutions as extractants of the P_2O_5 of alpha phosphates, with special reference to the relation between the "solubility" of the materials and their chemical composition. The official citrate and citric acid procedures were used.

(4) The "solubilities" of alpha phosphates in neutral ammonium citrate and 2 per cent citric acid solutions are not increased significantly by grind-

ing the materials finer than 200 mesh. The "solubilities" of 100-mesh samples are lower by 2 to 4 per cent of the total phosphorus.

(5) In "solubility" determinations on 18 samples of domestic types and sources of phosphate rock and macrocrystalline fluorapatite, including the parent materials of the current productions of alpha phosphate, about 5 to 13 per cent of the total P_2O_5 was extracted from the phosphate rock by neutral ammonium citrate and only 2 per cent from the fluorapatite. Two per cent citric acid extracts approximately two to four times as much P_2O_5 from natural calcium phosphate as does neutral ammonium citrate.

(6) The quantities of P_2O_5 extracted from *alpha* tricalcium phosphate and silicocarnotite, the latter being a less prominent constituent of at least some samples of alpha phosphate, by 2 per cent citric acid are only slightly higher than those extracted by neutral ammonium citrate. Neither reagent dissolves all the P_2O_5 from pure *alpha* tricalcium phosphate when the ratio of sample to extractant is 1 gram per 100 ml. Hydroxylapatite, a compound which has low fertilizing value and which is probably formed as an intermediate in the preparation of alpha phosphate, is more than 50 per cent soluble in citric acid but only about 25 per cent soluble in ammonium citrate.

(7) The "solubility" of partially defluorinated rock begins to increase when the fluorine content lies in the range corresponding with P_2O_5 to F ratios of 11.2 to 22.4. Since the products may contain three phosphate constituents—*alpha* tricalcium phosphate, glass, and apatite—in variable proportions, "solubility" behavior does not afford a valid indication of the variety of apatite present.

(8) The citrate solubility of alpha phosphate of relatively low fluorine content (0.5% or less) ranges from about 63 to 95 per cent of the total phosphorus. The different types of material, sintered, fused, and calcined, show about the same ranges in "solubility."

(9) Citric acid solubility is usually, but not always, higher than the citrate solubility. In the case of alpha phosphate with less than 0.5 per cent of fluorine, the average difference for 33 samples is 3.4 per cent of the total phosphorus.

ACKNOWLEDGEMENT

The authors are indebted to the several phosphate producers who supplied samples and to J. G. Cady, of this Bureau, who made the optical examination of the materials.

RECOMMENDATIONS*

It is recommended—

(1) That an analysis be made of the published results of plant-growth

* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 42 (1947).

experiments and tests with the various types of alpha phosphates (sintered fused, and calcined products), in order that a better understanding may be had of the fertilizing values of such products in comparison with those of superphosphate and other standard sources of P_2O_5 under a wide variety of soil, crop, and climatic conditions.

(2) That further study be made of the nature of the citrate-insoluble constituents of sintered, fused, and calcined alpha phosphates.

(3) That a study be made of the aging of the molybdate solution used in the volumetric method for P_2O_5 to see if a time limit should be put on its use or an addition made to preserve it.

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ANNOUNCEMENT

Referee Assignments, Changes, and Appointments

FERTILIZERS:
COPPER AND ZINC:
H. J. Webb, A. & M. College of South Carolina, Clemson, S. C., *vice* W. Y. Gary.
BORON:
G. N. Tyson, Jr., Pacific Coast Borax Co., Los Angeles, Calif., *vice* Stacey B. Randle.

PROCESSED VEGETABLE PRODUCTS:
QUALITY FACTORS:
B. Gutterman, Food and Drug Administration, Washington 25, D.C., *vice* Samuel C. Oglesby.
PEROXIDASE IN FROZEN VEGETABLES:
M. A. Joslyn, College of Agriculture, University of California, Berkeley 4, Calif.

COFFEE AND TEA:
Mary E. Ruffe, Food and Drug Administration, Chicago 7, Ill., *vice* H. J. Fisher.

PRESERVATIVES AND ARTIFICIAL SWEETENERS:
DICHLOROACETIC ACID:
John Thomas, Food and Drug Administration, New Orleans 16, La., *vice* A. Bruening.
THIOUREA:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C., *vice* H. I. Macomber.
DULCIN:
L. C. Andrews, State Department of Health, New Orleans 7, La.

ALCOHOLIC BEVERAGES:
DIASTATIC ACTIVITY AND SOLUBLE STARCHES:
Subject discontinued.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
MAPLE FLAVOR CONCENTRATES AND IMITATIONS:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.

FRUITS AND FRUIT PRODUCTS:
FRUIT AND SUGAR IN FROZEN FRUIT:
Paul A. Mills, resigned.

GUMS IN FOODS:
MAYONNAISE AND FRENCH DRESSING:
E. W. Coulter, Food and Drug Administration, Chicago 7, Ill.
STARCHY FOODS:
Sutton Redfern, resigned.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:
MERCURY:
H. F. O'Keefe, Food and Drug Administration, Chicago 7, Ill.

SYNTHETIC DRUGS:
PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS:
H. Rogavitz, Food and Drug Administration, New York, N. Y.

MICROBIOLOGICAL METHODS:
CANNED TOMATOES AND OTHER ACID VEGETABLE AND FRUIT PRODUCTS:
B. A. Linden, deceased.

CEREAL FOODS:
PROTEOLYTIC ACTIVITY OF FLOUR:
Sutton Redfern, resigned.

CORRECTION—MAY JOURNAL

In the paper on "Review of Methods for Detection of Caramel Coloring in Wine and Other Alcoholic Liquors," by Peter Valaer, published in the preceding number of *This Journal*, **30**, p. 335, 6th paragraph, 9th line, the word "not" should be omitted; the sentence should begin as follows: "This final solution, if colored, is submitted to the 'confirmatory test—tentative,' etc.

CONTRIBUTED PAPERS

THE DETERMINATION OF SUGAR IN BREAD*

By R. M. SANDSTEDT and J. C. FLEMING (Department of Agricultural Chemistry, Nebraska Agricultural Experiment Station, Lincoln, Nebr.)

The tentative method for sugars in bread and other bakery products as given in *Methods of Analysis, A.O.A.C.*, 1945, involves the extraction of sugars with boiling 50% alcohol and subsequent clarification with lead acetate. This procedure is not satisfactory on products containing any considerable quantity of protein soluble in 50% alcohol[1]; and the proteins of bakery products are not rendered insoluble in boiling 50% alcohol by the baking procedure. Since water extraction, with subsequent clarification with acid tungstate and determination of sugars by the ferricyanide method, has proved satisfactory for the determination of sugars in flour[2] it would seem to be the logical method for use with products containing wheat constituents. To determine its suitability for use with bread the method as used for the determination of sugars in flour was applied to the determination of known quantities of maltose and sucrose which had been added to bread crumb. Bread crumb of low sugar content was baked from doughs that had been largely depleted of sugar by fermentation. The crumb was dried in a vacuum oven at 70°C. and then ground in a burr mill. Samples equivalent to 5.2 g of fresh bread crumb,[3] 3.22 g dry weight, were placed in 100 ml Erlenmeyer flasks, aliquots of solutions of maltose, of sucrose, or of both maltose and sucrose were added, and the samples redried. This procedure gave samples containing known quantities of added sugar which simulated a series of breads varying widely in reducing and non-reducing sugar content.

SUGAR DETERMINATION

The reagents used for the determination of sugars were the same as those specified for the determination of sugars in flour.[2]

Fifty ml of the acid-buffer solution were added to the 3.22 g of prepared dry bread crumb, and the crumb suspended by shaking the flask. Two ml

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[1] Blish, *J. Biol. Chem.*, **33**, 551 (1918); R. M. Sandstedt, *This Journal*, **22**, 535 (1939).

[2] *Methods of Analysis, A.O.A.C*, 1945; **20.28, 20.29, 20.30.**

[3] The weight of dry bread crumb that may be used is limited by the amount of sugar that can be oxidized by the ferricyanide reagent; in general the sugar in approximately 3 g of dry commercial bread crumb will fall within the required limits.

If it is desired to report sugars on the fresh bread basis (using 38% moisture as standard) 3.22 g of dry bread should be used. The sugar per 10 g fresh bread may then be ascertained by direct reading from the "Maltose-Sucrose Conversion Table" as given for flour (*Methods of Analysis*, **20.30**).

On the other hand, if it is desired to report sugars on the dry weight basis, 3.12 g of dry bread should be used. The sugar per 10 g dry bread may be obtained by multiplying by 5/3, the reading from the "Maltose-Sucrose Table."

of the sodium tungstate solution were added, and the suspension thoroughly mixed. The mixture was filtered, and 5 ml aliquots of the filtrate were used for the determination of reducing and non-reducing sugars (as directed for flour).[2]

RESULTS

Table 1 gives the data obtained from dried bread crumb to which known amounts of maltose and known amounts of sucrose had been added. Each figure given is the average of 5 replications. The standard deviation is given for each set of replicated determinations.

TABLE 1.—*Recovery of maltose and of sucrose added to dry bread crumb*

	SUGAR ADDED	AMOUNT[2] DETERMINED	RECOVERY OF ADDED SUGAR		STANDARD DEVIATION
	mg/10g[1]		*mg*	*per cent*	
Maltose	None	81.4	—	—	.75
"	50	130.5	49.1	98.2	.71
"	100	179.4	98.0	98.0	1.3
"	200	286.2	204.8	102.4	1.5
"	300	378.4	297.0	99.0	1.7
"	400	493.2	411.8	102.9	1.2
Sucrose	None	16.2	—	—	1.8
"	75	89.8	73.6	98.1	1.5
"	150	163.7	147.5	98.3	1.1
"	225	237.0	220.8	98.1	.87
"	300	310.6	294.4	98.1	.55

[1] Fresh bread (38% moisture) basis.
[2] Averages of 5 replications.

TABLE 2.—*Recovery of mixtures of maltose and sucrose added to bread crumb*

MALTOSE				SUCROSE			
AMOUNT ADDED	AMOUNT RECOVERED[2]		STANDARD DEVIATION	AMOUNT ADDED	AMOUNT RECOVERED[2]		STANDARD DEVIATION
mg/10g[1]	*mg*	*per cent*		*mg/10g*[1]	*mg*	*per cent*	
50	48.8	97.6	1.1	37.5	36.9	98.4	1.1
100	98.0	98.0	.70	75	74.0	98.6	.68
150	147.0	98.0	1.7	112.5	109.9	97.6	1.2
200	195.4	97.7	1.6	150	146.4	97.6	1.2

[1] Fresh bread (38% moisture) basis.
[2] Averages of 5 replications.

Table 2 gives the data obtained from dried bread crumb to which had been added known mixtures of maltose and sucrose.

Table 3 gives the reducing sugar and non-reducing sugar found in two

samples of commercial bread. The two sets of data from bread sample #2 indicate that the sugars of bread are readily dissolved from the dried crumb; the shortest possible period of extraction gives maximum results, but, with a somewhat higher standard deviation than the longer extraction.

The above data indicate that the method is satisfactory. However, since the data were obtained on bread crumb which contained no milk it

TABLE 3.—*Sugar determinations on commercial breads*

BREAD	REDUCING SUGAR AS MALTOSE		NON-REDUCING SUGAR AS SUCROSE	
	AMOUNT	STANDARD DEVIATION	AMOUNT	STANDARD DEVIATION
	mg/10 gm[1]		*mg/10 gm*[1]	
1	542[2]	1.57	38.0[2]	2.1
2	649[3]	2.95	15.3[3]	2.4
2	653[4]	1.94	15.5[4]	1.8

[1] Fresh bread (38% moisture) basis.
[2] Averages of 10 replications.
[3] Averages of 5 replications—extract clarified and filtered immediately after adding acid-buffer to crumb.
[4] Averages of 5 replications—extracted 1 hr. before clarification.

is recommended that similar data be obtained on bread crumb containing 6 to 12 per cent of dry milk solids (lactose would be determined with maltose and dextrose as a reducing sugar). It is recommended also that similar data be obtained on bakery products containing eggs.

THE APPLICATION OF THE DITHIZONE METHOD TO THE DETERMINATION OF LEAD IN COAL-TAR COLORS*

By NATHAN ETTELSTEIN (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The determination of lead by the "dithizone" method has been investigated by a number of authors and has been shown by Clifford[1] and Wichmann[2] to give satisfactory results for lead in food and biological materials. Their work has resulted in the adoption of the tentative methods of the A.O.A.C.[3] The present study was undertaken to establish the applicability of these methods to coal-tar colors.

The samples of color were digested with sulfuric, nitric, and perchloric acids. The lead was then isolated as the dithizonate from the solution and determined by the electrolytic and titration procedures of the A.O.A.C.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 14–16, 1946.
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[2] Clifford, P. A., and Wichmann, H. J., *This Journal*, XIX, 130 (1936).
[3] *Methods of Analysis, A.O.A.C.*, 1945, 29.34.

PROCEDURE

REAGENTS

(a) *Standard lead solution.—Methods of Analysis, A.O.A.C.*, Sixth Edition, **29.36(a).**

(b) *Nitric acid solution, 1%.—Ibid.*, **29.36(b)**

(c) *Citric acid solution, 50%.—(Special grade—low in lead) Ibid.*, **29.36(d).**

(d) *Diphenylthiocarbazone (dithizone) solution.*—Stock solution of purified dithizone containing 100 mg. per ml. in chloroform. A working solution containing 20 mg. per liter. *Ibid.*, **29.36(e).**

(e) *"Stripping" reagent.—Ibid.*, **29.36(f).**

(f) *Potassium iodide solution, 2%.—Ibid.*, **29.36(g).**

(g) *Starch solution, 0.5%*—Weigh 1 gm. of soluble starch. Make into a thin paste with several ml. of cold water, pour into 200 ml. of hot water, and while still hot add 2–3 small crystals of HgI_2 as preservative.

(h) *Sodium thiosulfate solution.*—Make a stock 0.1 *N* $Na_2S_2O_3$ solution. Use 5 ml. isoamyl alcohol per liter as preservative. Prepare daily a fresh 0.001 *N* dilution and standardize against standard lead solution.

(i) *Potassium cyanide solution, 10%.*—Dissolve 50 gm. phosphate-free KCN in distilled water and dilute to 500 ml.

(j) *Hydroxylamine hydrochloride solution, 10%.*—Dissolve 10 gm $NH_2OH \cdot HCl$ in 20 ml. of water and make slightly alkaline with ammonia. Extract lead with dithizone. Remove excess dithizone with chloroform and boil off any $CHCl_3$ remaining in the aqueous phase. Acidify with HCl and dilute to 100 ml.

(k) *Thymol blue indicator solution, 0.1%.*—Dissolve 0.1 gm thymol blue in distilled water, add sufficient 0.1 *N* NaOH to change the dye to blue and dilute to 100 ml.

DETERMINATION

Transfer a 5.00 gm. sample to a 500 ml. Kjeldahl flask, add 10 ml. concentrated H_2SO_4 and 10 ml. concentrated HNO_3, and heat. When evolution of SO_3 fumes begins add 5 ml. of concentrated HNO_3 and heat until SO_3 is again evolved. Repeat the additions of concentrated HNO_3 each time SO_3 fumes appear until the dye is completely in solution and the digest is yellow. Then add 10 ml of 1:1 mixture of concentrated HNO_3 and 60–70% $HClO_4$; continue heating until the wet ash is colorless or pale yellow and the bulk of the H_2SO_4 is evaporated.

Cool the flask under running water and neutralize the solution by additions of small portions of concentrated NH_4OH. Add 20 ml. of the citric acid solution and then adjust to *p*H 8.5–9 with NH_4OH using four drops of thymol blue indicator. Add 5 ml. of 10% KCN solution.

Transfer the alkaline solution to a 250 ml. separatory funnel. Extract the lead with a 20 ml. portion of dithizone solution containing 20 mg. per liter. (*Note:* If there is sufficient iron present to cause excessive oxidation of the dithizone as indicated by a yellow color in the $CHCl_3$ layer, 10 ml. of 10% $NH_2OH \cdot HCl$ should be added to reduce the iron.) Allow the chloroform layer to settle and draw off into another separatory funnel. Wash down the floating globule of chloroform with two successive 5 ml. portions of weak dithizone (4 mg. per liter) and add to the receiving funnel. Repeat the extractions with the stronger dithizone until no more of the red lead dithizonate is observed. Do two more extractions with 10 ml. portions of the weaker dithizone solution.

Wash the chloroform extract with 25–30 ml. of distilled water containing one drop of concentrated NH_4OH. Draw off cleanly the washed chloroform layer into a third separatory funnel. Add 110 ml. of 1% HNO_3 and shake for one minute. Draw

TABLE 1.—*Recovery of lead in samples of coal-tar colors*

ADDED LEAD	BLANK*	TOTAL	RECOVERY	PER CENT RECOVERY
p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	
4	5	9	9	100
			8	89
			9	100
			9	100
8	6	14	14	100
			14	100
			14	100
			14	100
			14	100
12	5	17	16	94
			16	94
			16	94
			16	94
			17	100
20	5	25	25	100
			25	100
			26	104
			26	104
			25	100
50	4	54	54	100
			55	102
			54	100
			54	100
100	4	104	103	99
			103	99
			101	97
			103	99
Average Recovery				99

* The blank determinations include both the lead in the color composite and in reagents from the same batches, and in the same amounts, as were used in each series of recovery experiments.

off and discard the chloroform and about 1 ml. of the acid layer. Insert a cotton plug into the stem of the funnel to filter the acid layer as it is withdrawn. Discard the first 3 ml of the filtrate. Electrolyze a 100 ml. aliquot of the filtrate as directed in **29.41**, *A.O.A.C. Methods of Analysis*, Sixth Edition.

Application of this procedure to composite samples of FD&C Yellow No. 5 showed 5 parts per million (including reagent blank). Known amounts of lead ranging from 20 to 500 micrograms (equivalent to 4 to 100 parts per million) were added to 5 gm. portions of the dye and then

determined by the outlined method. Per cent recovery is shown in the accompanying table.

SUMMARY

Recoveries of known amounts of lead in coal-tar colors averaged 99 per cent. It is believed that the accuracy of the method is limited only by the precision with which the blank can be determined.

A GLASS ELECTRODE ASSEMBLY FOR SOIL *p*H DETERMINATION[1]

By C. J. SCHOLLENBERGER (Associate in Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio)

In determining soil *p*H, the values obtained tend to vary directly with the ratio of water to soil in the sample as prepared for examination. With increasing dilution there is increasing hydrolysis of potentially basic soil constituents and decreasing hydrogen exchange and ionic strength, all operating to increase *p*H. Extreme differences have been noted with western irrigated soils (9, 12, 14), and even with humid soils they are sufficient to make desirable the determination as nearly at field moisture contents as possible (6, 15). The thin spherical bulb glass electrode furnished with a well-known *p*H meter cannot safely be thrust into moist soil firmer than a thin paste. For that reason, soil with 100 per cent water (1:1 ratio) is recommended for the determination (15, 16), although the desirability of a water content not beyond "saturation" is recognized (1, 13, 14, 17). At present, the manufacturers supply only this type of electrode, which is necessarily expensive from its excellent construction and impractical to repair if broken, because of its sealed-in-a-unit design. The electrodes of some other manufacturers are more rugged, and could be used with this *p*H meter, but none of the commercial electrodes is particularly well adapted to use with soil save in an open container, nor would any be easy to use in a flask, as might be desirable, for example, in soil-CO_2 equilibrium studies. There is need for a glass-and-calomel half cell electrode assembly designed especially for work with soils; sturdy, inexpensive, and which may be constructed in desired dimensions or repaired by the user.

In Figure 1 is shown the author's design for a spear type glass electrode assembly. The *p*H-sensitive bulb is shaped to present a large surface for contact with the sample, to be strong without excessive thickness at the center of the area otherwise weakest, to press with increasing firmness against the sample as it penetrates to ensure close contact therewith, and

[1] Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio.

to penetrate moist soil with minimum liability of fracture. A bulb of this form is almost as easily blown from tubing of Corning 015 electrode glass[2] as a spherical bubble; and the absence of the usual seal to soft glass

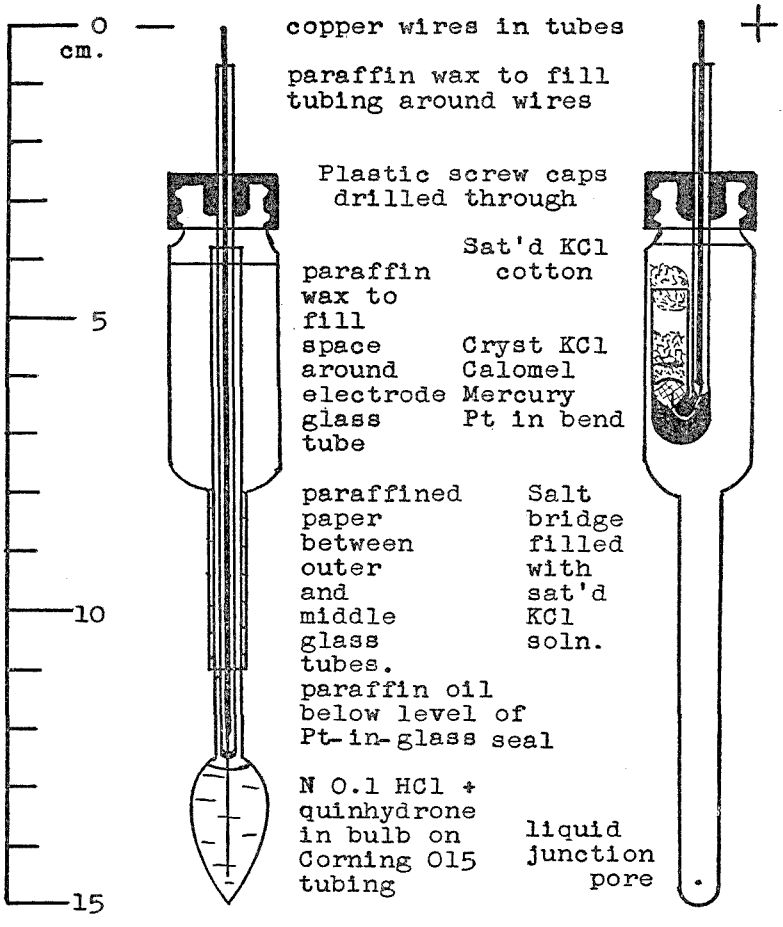

FIG. 1.—Glass electrode assembly

facilitates construction of the half cell by one slightly skilled in glass working. The design permits easy replacement of the bulb if broken, and reassembly to the same or any desired dimensions. These features seem particularly important in soil work, since repeated penetration of the soil mass by the bulb is advised for "conditioning" the latter, to hasten attainment

[2] Obtainable from Corning Glass Works, Corning, N. Y. A recent quotation was $4.00 per pound.

of equilibrium; and a large surface in close contact should give a better mean value for *p*H, which has been shown to be a resultant of local values at points of contact (4). The drawing is to scale for a half cell 15 cm in length, a convenient size for general purposes. The half cells may be made longer or shorter, as may be desirable for special uses; the electrode glass bulb may be larger or smaller; and the stem (salt bridge) of the calomel half cell may be bent to bring stem and bulb into close proximity so that both can be inserted into the mouth of a flask for a determination on the contents without exposure to the atmosphere. The construction is simple and should be plain after studying the figure; some parts will be described in detail.

CONSTRUCTION OF PARTS

The bodies of both half cells are made from "applicator vials" with molded plastic screw caps, each carrying internally a glass rod cemented in a boss and easily removed.[3] The bottoms of the vials are drawn to a taper and cut off at the proper point, and suitable glass tubes sealed on. For the glass half cell, this tube should be of slightly greater diameter internally than the electrode glass tube is externally, so that a layer or two of paraffined paper will fill the space between them. This tube is afterwards cut off about 30 mm from the seal. For the calomel half cell, the tube sealed on should previously have been cut to the proper length, after closing its lower end and providing it with a pore for the liquid junction, as will be described.

The spear type bulb for the glass electrode is blown thus:

Wash with dilute hydrochloric acid, rinse and dry a length of Corning 015 electrode glass tubing about 6 mm in external diameter. Heat a narrow zone and draw straight apart to obtain a sealed tube several inches longer than the desired finished length. Hold this sealed end at an angle in a good-sized, hot Bunsen flame, with constant turning while supporting the thin tail with the other hand, until about 12 mm of the full diameter portion is well softened, the wall slightly thickened, and the glass cleared of any devitrification resulting from the drawing. The heating must be rapid and even on all sides.[4] Remove from the flame, hold perpendicular with the heated end down, grasp the thin tail, draw downward axially, and simultaneously start blowing with increasing force while continuing the draw in order to elongate the bulb. The blowing must be completed in about two seconds, without reworking or annealing. The result should be a streamlined ovoid about 25 mm long and 12 mm maximum diameter, terminating in a sharp point at the thin thread below. Melt this thread off at the point of the bulb, and round a little over a small flame. A good bulb should be symmetrical, of the shape desired, without defects in the glass, and not too thin. It should be able to stand moderate pressing between the fingers, or pressing axially into fairly stiff moist soil. If the first bulb is defective, melt it down and draw off *all* the spoiled glass; then blow another on the tube. When a bulb that

[3] These vials are listed by dealers, *e.g.*, Fisher—E. & A., Cat. No. 3-339, at $0.70 per dozen. The capacity is 8 ml, diameter 19 mm, length 50 mm, neck opening 10 mm. They are nicely made of flint tubing to which ordinary soft glass tubing may be sealed.

[4] An unsymmetrical bulb will result from uneven heating. The electrode properties of the glass are easily injured by excessive or prolonged heating, probably from expulsion of essential water and sodium. Damaged glass shows a dull or wrinkled surface, or opaque white spots.

looks good is secured, it is advisable to test it by filling with quinhydrone-saturated decinormal hydrochloric acid only, inserting the internal element, and testing in buffers as will be described. Some lengths of tubing have not produced satisfactory bulbs. For final assembling, cut the tube to proper length, clean and warm it, and apply a thin even coat of paraffin wax. Wrap with paraffined paper and insert into the tube sealed to the vial, so that the open end will be near the mouth. Fill the space in the vial with melted paraffin, for additional security and insulation.

The pore for the liquid junction in the tube to be used for the calomel half cell is formed thus:

Heat the sealed end moderately, uniformly, and just to the softening point at a spot about 5 mm from the bottom. Bring into the flame a blunt pointed glass rod, press lightly to the heated spot until it adheres and withdraw from the flame. Immediately pull away the rod, radially with respect to the tube diameter, thus drawing from the tube a thread of glass hollow at its base. Cut this off as close to the tube as possible. If the top of the stump is irregular, or the cut is above the hollow portion, grind down. The hole produced will probably be too large; clean and dry the tube and heat to shrink the pore. At intervals, let cool sufficiently to harden the glass, hold in a luminous flame and blow to judge the size of the pore. It should be made as small as possible, without the risk of having it close entirely. Cool the tube and blow into water; single small bubbles should escape, showing that there is an opening. Blow into the water filled tube; no drop should fall quickly. The aim is to secure a circular and thin-edged pore about 10 microns in diameter. A liquid junction through such an opening seems to satisfy the requirements outlined by Dole (5), and is perhaps the nearest approach to the ideal flowing junction[5] that is practical in working with soil. It is used on some commercial calomel half cells. If made small as directed, diffusion of the salt solution into water is no greater than with a tightly fitting ground glass plug (18): about 0.003 ml saturated potassium chloride in 10 minutes.

The internal element for the glass half cell is a 25 mm length of thin platinum wire or of stiff platinum foil one mm wide, annealed and straightened after cutting with shears. To this is welded a length of bare No. 20 copper wire, thus: With pliers hold the wire, with about 15 mm of one end free. Moisten and dip into powdered borax, and heat to give a thin even coat of borax glass. With the wrists pressed together for steadiness, hold the platinum by tweezers in the other hand, ready to bring into the flame in line with the copper wire. Heat the end of the copper wire in a small blast flame and, at the instant the copper fuses to a slight bead, bring the two metals together and withdraw them from the flame in contact. A neat and perfect weld should result. Crack off the borax glass and dissolve adhering fragments with hot water. Push the welded end through a 3–4 mm diameter glass tube[6] of suitable length until about 15 mm of the platinum projects from the other end. Melt the end of the glass tube around the platinum, taking care that the weld is well out of the seal. Soot a little, and cover with a dry cloth after removal from the flame, to retard cooling and lessen the risk of cracking the seal.

For the calomel half cell internal element, join a short piece of 5–6 mm and a longer piece of 3–4 mm diameter tubing,[6] and cut off the larger tube 30 mm from

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[5] Ground glass plug and shell, or wick, liquid junctions are undesirable because the zone of diffusion may become very wide, with resulting high resistance and no reproducibility. But the maximum error to be expected from this cause will seldom exceed one millivolt, practically the limit of sensitivity of *p*H meters.

[6] Machine-made flint tubing made by Kimble Glass Co., Vineland, N. J., has been recommended as best for sealing around platinum (10). Pyrex tubing may be used if the platinum sealed in is of very small cross section and the seal is made thick and strong, as with that last described. Pyrex, of course, requires an oxygen-fed blast flame.

the seal. Weld to copper wire the broader end of the narrowest possible 20 mm sliver of platinum that can be cut from foil. Push the weld into the smaller tube until the middle of the platinum is under the seal. Melt together the glass here and work in enough from the adjacent tubing to make a solid U bend with the tubes in contact, to enter the mouth of the vial, and keeping both ends of the platinum out of the seal.

Warm the upper ends of the 3–4 mm tubing of both internal elements, heat the exposed ends of the copper wires and hold against a cake of paraffin so that the melted wax runs down the wires to fill the space around them. When solidified, this will cement the wire in place and protect the thin platinum and seal from strains; it will also keep the electrolyte away from the copper if the seal is imperfect, besides furnishing additional insulation.

Remove the glass rods from the molded plastic screw caps of the applicator vials and drill into the holes in which the rods were cemented, and through the top of each cap. Ream out the holes to take the 3–4 mm glass tubes of the internal elements, and cement the latter with "Duco" in proper position as shown in Figure 1.

FILLING THE ELECTRODE VESSELS

Shake decinormal hydrochloric acid with a small excess of quinhydrone crystals to saturation. Drop about 0.01 g of the dry crystals into the bulb of the glass half cell, and with a thin-stemmed pipet, wiped dry externally, nearly fill the bulb with the acid solution. Add sufficient U.S.P. paraffin oil to come nearly to the top of the electrode glass tube when the internal element is in place. Wet the platinum of the element with a drop of the remaining acid and plunge quickly through the oil, well into the acid in the bulb, but with the seal still in the oil layer, and tighten the cap on the vial. When not in use[7] suspend the filled half cell in a test tube with sufficient distilled water to cover the bulb, and protect from light.

Prepare a solution of potassium chloride, 40 parts to 100 of water, and let cool to room temperature. With a thin rod suitably bent, push down the projecting platinum in the upturned open tube of the internal element for the calomel half cell, into close contact with the seal. Add a large drop of pure mercury and heat[8] carefully to boil the mercury for an instant, thus expelling entrapped air and ensuring perfect contact. Add a 2–3 mm layer of electrolytic calomel and a few drops of the potassium chloride solution, and stir well to remove air bubbles. Follow with a layer of potassium chloride crystals,[9] stir and finally fill the open end of the tube with the solution. Hold the internal element in a small beaker containing sufficient solution to cover the filled tube, drop in a bit of dry absorbent cotton and work air bubbles from it before stuffing into the tube with the aid of the rod. The cotton is to hold the filling in the tube, in case the half cell should later be inverted. Pour enough of the solution into the vial to fill, with the internal element in place, insert the element

[7] The oil is for insulation: considered desirable because the electrode glass is more surface conductive than ordinary glass. The oil covering may help protect the quinhydrone from oxidation, but it does not prevent the gradual darkening of the solution and the separation of insoluble decomposition products, believed to be accelerated by exposure to light. In the author's experience, this has happened in a commercial electrode of sealed construction excluding air. Nevertheless, this electrode was satisfactory in performance for several years after it had become unsightly. Any change in the potential of the internal element so caused should be gradual and compensated by the asymmetry correction for the electrode. To clean an old electrode, or before blowing a new bulb on the shank to replace one broken, it will be necessary to remove the resinous decomposition products and oil by heating in a test tube containing concentrated sulfuric acid with some nitric acid.

[8] This is not likely to crack the seal if Pyrex glass was used in the construction.

[9] Recommended by Bureau of Standards workers (19), although apparently contrary to a statement by Dole (5). In a private communication, the latter advises that there is no objection to solid potassium chloride in the half cell or salt bridge, if the crystals are *wet* with the solution. But if allowed to dry, as may happen when the pore is left exposed to the air indefinitely, the insulating effect of dry crystals may prevent the electrode from functioning. Crystals in the salt bridge, even if above the level of the pore, are harmless and in fact advantageous, as they are visible evidence that the solution is saturated, ensuring minimum error from junction potential (5).

and tighten the cap. When not in use, keep the filled calomel half cell standing in a test tube with enough saturated potassium chloride solution to cover the pore. A coating of paraffin wax on the upper interior of the test tube, and on the exterior of the half cell, will lessen annoyance from creeping salt.

MOUNTING

The slender design of the half cells permits close mounting of the electrode assembly. If made short the assembly can be used in the enclosed compartment of a *p*H meter. Short lengths of heavy rubber tubing may be split for attachment to the stems, for gripping by, and insulation from, the metal clamps. A separate electrode holder is preferable if many determinations are to be made. A small block of hardwood to effect the desired separation can be bored at one end to take a support rod, and a thumbscrew for tightening. Near the other end along both sides of the block, are bolted strips of bakelite panel board. Similar strips with matching grooves are loosely attached by pins to hold the half cell stems when they are tightened thereon by a small bolt with thumbnut. The assembly can be lifted from position over a beaker containing the sample and lowered again after shifting the beaker slightly, so that the spear bulb and salt bridge tip penetrate the mass at a different place, thus affording a confirmatory reading. Connection to the meter may be made by the commercial shielded cables, or by radio hook-up wire sufficiently stiff to stand in position.

TESTING THE GLASS ELECTRODE

*p*H meters are designed with the assumption that the EMF-*p*H relation of the glass electrode conforms to theory, *i.e.*, the slope is strictly in accordance with the Nernst electrode equation. The manufacturers of glass electrodes doubtless sell only those which have passed their tests, but here again it is assumed that an electrode may not change in its characteristics. Both assumptions are unsafe and there is a possibility of considerable error if the *p*H of the sample is far from that of the buffer used for setting the meter for "asymmetry" of the electrode. The magnitude of such error will be less if the *p*H of the buffer is near that of the sample. From information in various research papers of the National Bureau of Standards, the following[10] are considered suitable for soil work:

m 0.05 potassium hydrogen phthalate, pH 4.00 (8).—Prepare a *m* 0.25 stock solution from 5.059 g of the pure dried salt, dissolved and diluted to 100 ml. Dilute a portion with 4 volumes of water for the standard.

m 0.025 disodium hydrogen phosphate and potassium dihydrogen phosphate, pH 6.86 (3).—Dry the powdered crystals of the former in an oven gradually heated to 130° C, to avoid melting in crystal water, and weigh out 1.775 g of the dried salt. Dissolve this and 1.701 g of the dried potassium salt, and dilute to 100 ml with CO_2-free water for a stock solution. Dilute a portion with 4 volumes of water for the standard.

[10] The chemicals, in highest purity, are sold by the Bureau as standard samples, with full directions for preparing buffer solutions of known *p*H values (2).

m 0.01 disodium tetraborate decahydrate, pH 9.15 (11).—Prepare a solution of purest borax, carbonate free, saturated at 60° C. Chill with constant stirring to obtain fine crystals. Filter on a Büchner funnel, wash twice with alcohol and twice with ether, spread to dry in the air until the ether has evaporated, and bottle. Dissolve 1.905 g of this decahydrate and dilute to 100 ml with CO_2-free water for a stock solution. Dilute a portion with 4 volumes of water for use. This buffer solution absorbs CO_2 when exposed to the atmosphere; the equilibrium *p*H is near 8.95, after several days aeration (7).

Adjustment for asymmetry potential at *p*H 4.00, followed by a reading in *p*H 9.15 borax buffer, or vice versa, is the test for excellence. Of about two dozen bulbs blown by the author in the manner described, about a third performed well in respect to coming to equilibrium quickly—in not over five minutes—and gave nearly theoretical readings in the second buffer. The best of these were made from one length of 6 mm diameter Corning 015 glass tubing. Of the four commercial spherical bulb glass electrodes available for comparison, only one was noticeably better than the best of those homemade. One of the commercial electrodes standardized at *p*H 4.00 gave readings initially too high in the borax buffer, decreasing to the correct value within a few minutes; another gave readings initially too low, but increasing to the correct value in about the same time. With still another, the indicated figure did not reach the correct value in any reasonable time. The latter behavior was characteristic of all the unsatisfactory homemade bulbs: set at *p*H 4.00 in biphthalate buffer, they read no higher than *p*H 8.7–.8 in borax buffer. The better ones reached the theoretical value, or very near it, generally within two or three minutes, and remained at that point; they never gave a reading higher than the correct one. It was noted that some of the bulbs performed better after standing in distilled water for several days; in other cases external treatment with dilute hydrochloric acid followed by ammonia effected some improvement. However, the latter treatment was not in all instances beneficial; one bulb, giving practically correct readings but covered with a cloudy film, was less accurate after an attempt at cleaning.

SUMMARY

Matched half cells for a glass electrode assembly to be used with a *p*H meter in examining wetted soil, etc., in a beaker or flask, are described, with details of their simple construction from readily obtainable parts. The spear type *p*H-sensitive bulb is easily blown from Corning 015 glass tubing, is not sealed to other glass but rather attached to permit replacement, and is shaped to give maximum strength and sensitivity and to penetrate the soil mass with minimum risk of fracture. The internal element is a quinhydrone electrode in decinormal hydrochloric acid. A pore type liquid junction in a combined salt bridge and saturated potassium chloride-calomel half cell (considered best for use with soils) is described. In per-

formance, this assembly has compared favorably with commercial glass electrodes. Formulas are given for buffer standards to be used for setting the meter and testing electrodes.

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IDENTIFICATION OF THE MONOHALOGEN SUBSTITUTED ACETIC ACIDS AS BARIUM SALTS

By WILLIAM V. EISENBERG and JOHN B. WILSON*

During the war, because of the shortage of sulfur dioxide, the French government permitted the use of monobromoacetic acid, as such or in the form of its sodium salt or ethyl ester, to preserve grape juice (1) and to check fermentation in the manufacture of wine (2) ("mutage"). Because of the possibility that this preservative, and perhaps the analogous iodine and fluorine compounds as well, might be encountered in imported juices and wines, the method of Wilson and Keenan (3) for identifying monochloroacetic as the barium salt was extended to the other monohalogen derivatives of acetic acid. Quantities of the various barium salts were prepared from about 100 mg of each of the several acids, as directed in the paper referred to above, and the crystals were examined under the polarizing microscope. The data obtained are given below.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF BARIUM MONOFLUOROACETATE†

In ordinary light, under the microscope, the anhydrous salt is observed

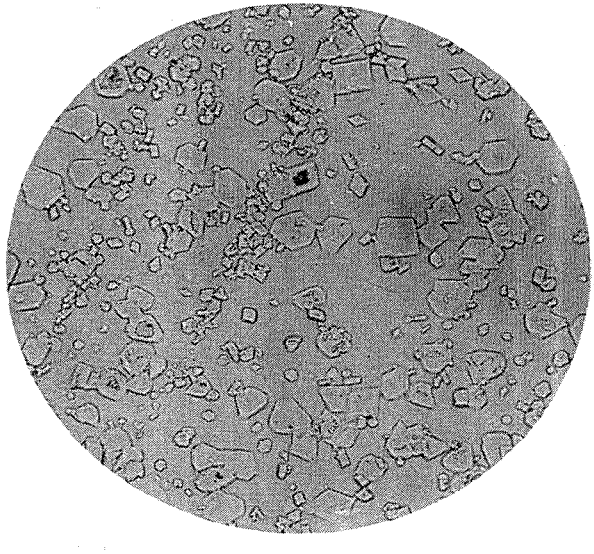

FIG. 1.—Barium Monofluoroacetate Anhydrous (250 ×).

* Joint contribution from Food Division (W. B. White, Chief) and Microbiology Division (G. C. Slocum, Acting Chief), U. S. Food and Drug Administration.

† Monofluoroacetic acid and its salts are deadly poisons and should be handled only with the greatest caution.

to crystallize from water as small monoclinic prisms with no definite elongation. The prisms are truncated, forming a variety of irregular polyhedrons (Fig. 1). In parallel polarized light (crossed Nicol prisms), the extinction is parallel and inclined to the various prism faces. The refractive indices, determined by the immersion method in organic liquids, are $n_\alpha = 1.520$, $n_\beta = 1.533$, and $n_\gamma = 1.578$ (all ± 0.002). In convergent polarized light an off-centered and indistinct optic axis figure is observed occasionally. The intermediate index n_β was therefore determined statistically.

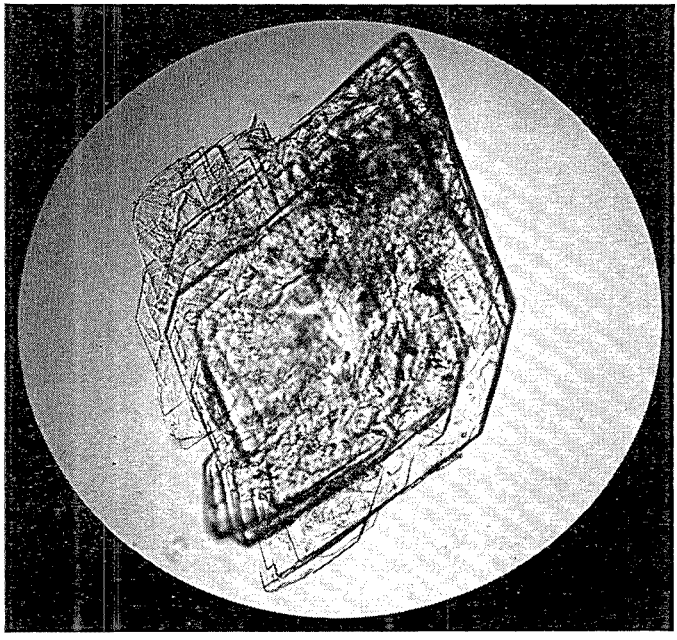

FIG. 2.—Barium Monochloracetate Monohydrate (100 ×).

A hydrated salt of barium monofluoroacetate may be obtained from water solution if the salt is allowed to crystallize slowly under humid conditions. The following procedure was used: A microscope slide with a drop of the solution was placed in a covered petri dish. After ½ to 1 hour the water had evaporated spontaneously, leaving crystals of a hydrated salt. The crystals develop as elongated prisms with inclined extinction. In convergent polarized light an optic axis figure with a very large axial angle is observed. The optic sign is indistinct but is probably negative. The refractive indices, determined by the immersion method in organic liquids, are $n_\alpha = 1.475$, $n_\beta = 1.498$, and $n_\gamma = 1.524$ (all ± 0.003). All three significant indices are readily measured.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF BARIUM MONOCHLOROACETATE

These properties, for the hydrated salt, are given by Wilson and Keenan (3) (see Fig. 2); those for the anhydrous salt are given by Eisenberg (4). The data are included in Table 1 for convenience.

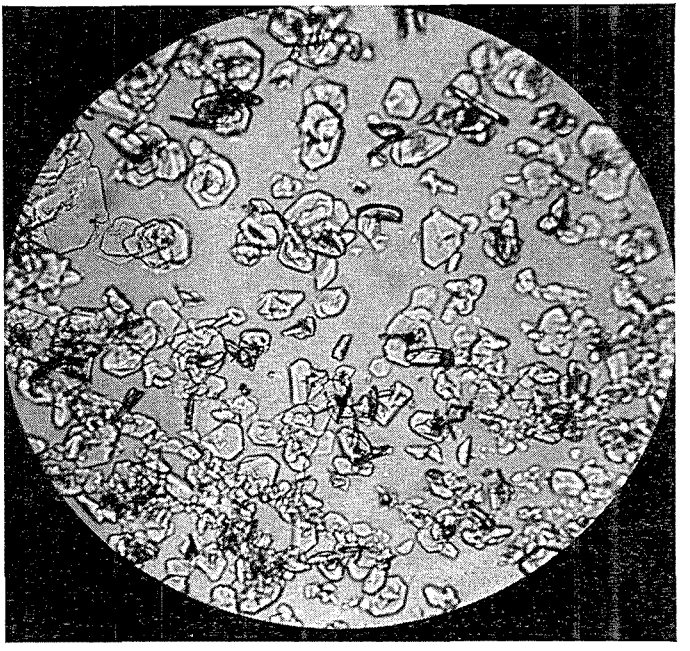

FIG. 3.—Barium Monobromoacetate Anhydrous (100 ×).

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF BARIUM MONOBROMOACETATE

In ordinary light, under the microscope, this salt is observed to crystallize from water as monoclinic prisms. The prisms are mostly truncated, exhibiting an irregular 6-sided outline (Fig. 3). In parallel polarized light (crossed Nicol prisms), the extinction is parallel, and the sign of elongation positive on the elongated prisms. No interference figures were observed in convergent polarized light. The minimum and maximum refractive indices were observed on prisms showing maximum birefringence. The two significant indices are $n_\alpha = 1.548$ (± 0.002) and $n_\gamma = 1.705$ (± 0.005). We were able to produce this salt only in the anhydrous form.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF BARIUM MONOIODOACETATE

In ordinary light, under the microscope this salt is observed to crystallize from water as monoclinic prisms. Many of the crystals show a rectangular outline and some are truncated, forming prisms with a 6-sided and 8-sided outline (Fig. 4). In parallel polarized light (crossed Nicol

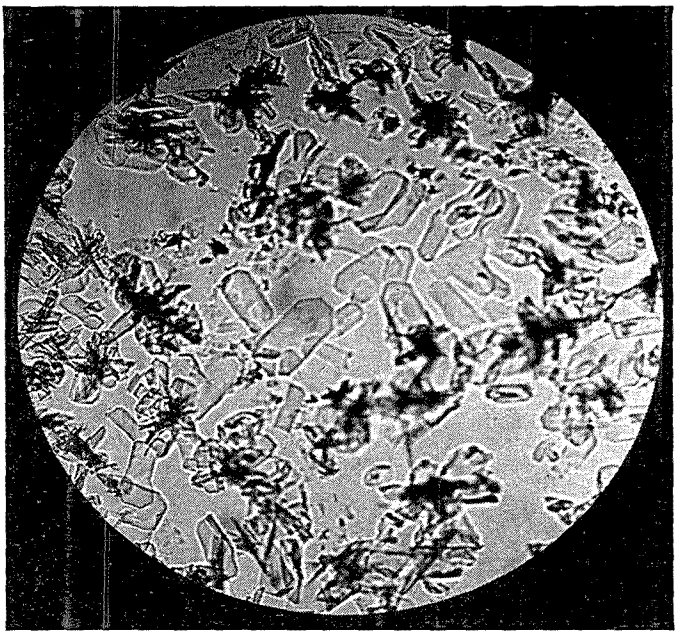

FIG. 4.—Barium Monoiodoacetate Anhydrous (100 ×).

prisms) the extinction is parallel, and the sign of elongation positive, on the elongated prisms. The refractive indices, determined by the immersion method in organic liquids, are $n_\alpha = 1.633$ (± 0.002), $n_\beta = 1.75$, and $n_\gamma = 1.85$ (both ± 0.01). In convergent polarized light an occasional optic axis figure with a large axial angle and negative optic sign is observed. The dispersion of the optic axes is strong. The three significant refractive indices are readily observed. The intermediate index, n_β, is obtained by fracturing the prisms and observing the particles which do not extinguish sharply but show an optic axis figure in convergent polarized light. As in the case of barium bromoacetate, only anhydrous barium iodoacetate has been obtained so far.

For the convenience of analysts examining unknown barium salts obtained by the recommended procedure, the optical crystallographic prop-

erties of the various salts are summarized in the following table, arranged according to the ascending value of the minimum refractive index.

TABLE 1.—*Optical-crystallographic properties of the barium salts of the monohalogen substituted acetic acids*

SUBSTANCE	SYSTEM & HABIT	N_α	N_β	N_γ	OPTIC SIGN & ANGLE	ORIENTATION
Barium monofluoroacetate hydrate	Elongated prisms	1.475	1.498	1.524	2V = very large	Ext. inclined
Barium monochloroacetate anhydrous	Mon. plates	1.512	—	1.638	—	Ext. par.; Elong. neg.
Barium monofluoroacetate anhydrous	Mon. prisms	1.520	1.533	1.578	2V = very small	
Barium monobromoacetate anhydrous	Mon. prisms	1.548	—	1.705	—	Ext. par.; Elong. pos.
Barium monochloroacetate monohydrate	Mon. plates	1.582	—	1.611	—	Ext. par.; Elong. neg.
Barium monoiodoacetate anhydrous	Mon. prisms	1.633	1.75	1.85	negative 2V = large	Ext. par.; Elong. pos.

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COMPARISON OF SEVERAL TYPES OF APPARATUS DEVISED FOR THE DETERMINATION OF VOLATILE OIL IN CITRUS JUICES[1]

By A. LAURENCE CURL (U. S. Citrus Products Station,[2] Winter Haven, Florida)

The determination of the volatile (peel) oil content is essential in judging the grade of canned grapefruit, orange, or blended citrus, juices. Several types of apparatus have been devised for this purpose, but little has been published on their relative efficiency and accuracy. Faults have been noted in some models. For example, in one apparatus commonly used in the Florida citrus industry the oil column in the trap tends to separate, and a portion often returns to the distillation flask or becomes lodged at the T below the buret. A comparative study was therefore made

[1] Agricultural Chemical Research Division Contribution No. 205.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

of several models now in use, and this led to the construction and testing of a number of new ones. Comparable recoveries of limonene from mixtures of limonene and distilled water were obtained with a number of models. The advantages and disadvantages of some of the models tested are discussed.

A few types of apparatus for the determination of volatile oil in citrus fruits, peels, or juices have been described in the literature. A number of others have been devised by chemists in the citrus industry and some of these have been manufactured commercially. C. P. Wilson and Young (6) described a method for the determination of volatile oil in finely ground whole citrus fruits. Scott (4) applied this method to the determination of volatile oil in grapefruit juice. The apparatus of Clevenger (2) or a modification of it has been used extensively for the determination of volatile oil in citrus juices. J. B. Wilson (7) modified the Clevenger apparatus so that the condensate did not fall directly into the oil in the trap, and also added a bulb below the buret to prevent any drops of oil from returning to the distillation flask. This apparatus is also described in the U. S. Department of Agriculture standards for grades of canned orange juice and canned grapefruit juice, recently issued (5). Bartholomew and Sinclair (1) in a method for the determination of volatile oil in citrus peels described a novel type of apparatus which is somewhat more complicated than the others mentioned above.

EXPERIMENTAL

Apparatus:

Thirteen models of seven different types were used in this investigation, but for simplicity only six models of three types are included in this report. These three types include two in common use at the present time, and another which the writer deems to be the most generally satisfactory of all tested. Only two of the seven types were unsatisfactory in that significantly lower results were obtained; other factors, such as ease of cleaning, fragility, simplicity, and speed of operation, were considered in comparing the various apparatuses.

The three general types of apparatus are designated by Roman numerals I to III, and minor modifications by Arabic numerals, as in I-1 and I–2. When a given model was used in two ways, the alternate is indicated by the letter *a*, as in I-2-a. One model had a micro-buret of 0.4 ml. capacity, indicated by the letters *mi*, as in III-mi. In the other models the burets were of 2.0 ml. capacity and are referred to as macro-burets. When a wetting agent (see below) was used in transferring the oil to the graduated portion of the buret for measurement, the model is indicated by the letter *w*, as in III-w.

In the figures, the distillation flasks are not illustrated. These were of either two- or three-liter capacity with the macro-buret apparatus, and of one-liter capacity with the micro-buret.

Model No. I-1.—(Fig. 1)—The most widely-used apparatus for the determination of the volatile oil content of citrus juices is probably a modification of the Clevenger apparatus (2). This differs from the original Clevenger apparatus in that the cold-finger condenser and buret have been so rearranged that the condensate does not drop directly into the buret. In model No. I-1 the water level in the filled trap stands about even with the top of the buret tube.

Model No. I-2.—This was similar to No. I-1, differing only in that the

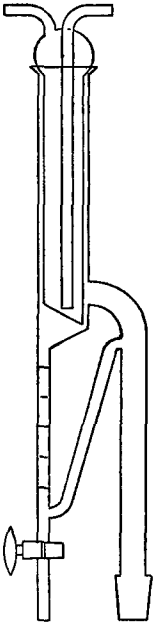

FIG. 1.—A commonly-used modification of the Clevenger apparatus (Type I).

water level in the filled trap stands slightly higher. In No. I-2-a the apparatus was tilted so that all of the oil collected above the buret tube; for measuring the oil the buret was made vertical.

Model No. I-3.—This was similar to Nos. I-1 and I-2, but was constructed so that all of the oil collected above the buret when the apparatus was vertical.

Model No. II.—This was a commercial model of the apparatus of J. B. Wilson (5, 7). As received the condenser was only 5 cm. long; therefore another one 13 cm. long was substituted. As this condenser fitted rather snugly, it was rinsed off with water at the end of the reflux period.

Model No. III.—(Fig. 2)—This was a modification of the apparatus developed by R. B. McKinnis (Model No. III-mi). The custom stopcock was

replaced by a small hemispherical joint, and a macro-buret was used in place of the micro-buret. Beneath the buret a piece of 11-mm. tubing 40 mm. long was sealed, the bottom being closed by a rubber stopper. The leveling tube was attached near the bottom of this tube. The McKinnis apparatus differed from the other types in that the inlet tube fitted loosely into, and extended about half-way down, the upright condenser,

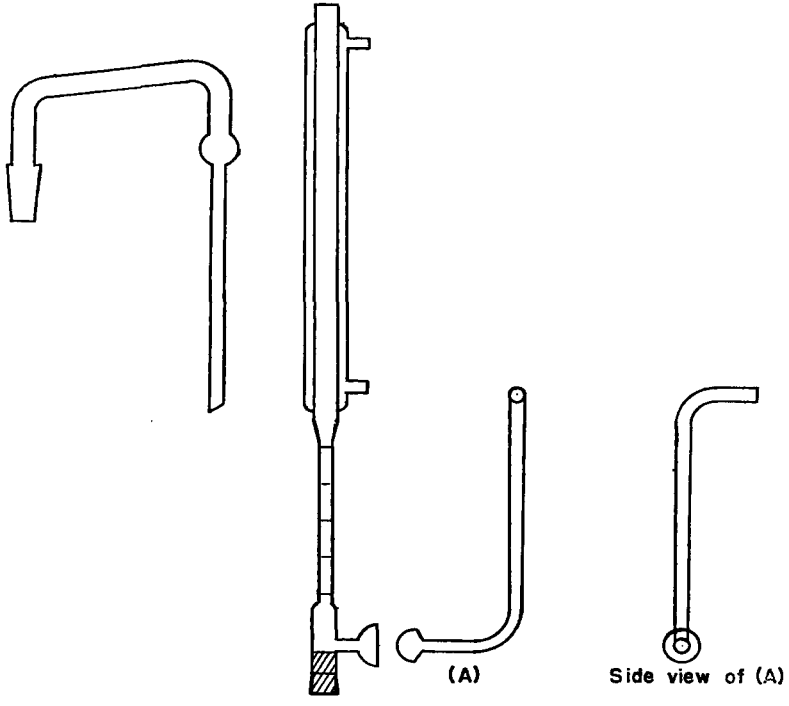

FIG. 2.—A modification of the R. B. McKinnis apparatus (Type III).

the vent being at the top of the condenser. The buret was attached below the condenser, and the water level was maintained in the wider tube above the buret during distillation by adjusting the leveling tube. Collecting the oil in this wider tube makes possible the use of a buret of smaller bore. The water distilled over escapes through the leveling tube and is not returned to the distilling flask. At the end of the distillation the flask and inlet tube are removed and the oil is lowered into the buret by adjusting the leveling tube. In *No. III-w* a wetting agent was used to rinse the condenser at the end of the distillation.

Model No. III-mi.—This was made according to the drawings obtained from R. B. McKinnis (3). A micro-buret was used, and the level of the

liquid in the trap was controlled by a leveling tube attached to a special stopcock just below the buret. It functioned like model No. III. The wetting agent was always used.

Other Models Tested.—With three other macro-buret models that were tested, the results were about the same as with Nos. II and III. Each of these models had some good features but on the whole No. III was considered preferable. One of these (No. IV) was similar to Model No. I (Fig. 1) but was equipped with a built-in Liebig condenser instead of a removable cold-finger condenser. The second (No. V) differed from No. IV in that the vapors from the distilling flask enter the top of the condenser, and a vent tube is provided between the condenser and the buret. The other (No. VI) was similar to Model No. III (Fig. 2) except that the connecting tube from the distilling flask was attached to the top of the condenser by means of a hemispherical joint, and a vent tube was provided between the condenser and the buret. Two other micro-buret models, similar to Nos. V and VI, were tested, but were found to be less satisfactory than No. III-mi.

Burets:

The buret in Model No. I-3 was made from a 2-ml. portion of a 5-ml. graduated pipet and that on No. III-mi from a 0.04-ml. portion of a 1-ml. graduated pipet. The 2-ml. burets on the others were part of, or obtained from, commercial peel-oil apparatus. The distance between 0.1 ml. graduations was 5.6 mm. in Nos. I-1, I-2, and III, 4.0 mm. in No. I-3, 3.4 mm. in No. II, and 15.0 mm. in No. III-mi.

As a check, the buret of each macro-buret apparatus was filled with water as in a determination, and 1.00 ml. of limonene was added, using the same 1-ml. graduated pipet as in all of the recovery studies. The limonene was then lowered into the buret and measured. The readings obtained ranged from 1.00 to 1.02 ml., hence the burets were used without applying any correction. In the case of the micro-buret model, No. III-mi, 0.250 ml. of limonene was used. Since the readings obtained were several per cent high, this procedure was repeated with 0.200, 0.150, 0.100, and 0.050 ml. amounts of limonene, two or more measurements being made for each value. The average results are given in Table 2.

Wetting Agent:

The wetting agent used was provided by Dr. McKinnis (3) and according to him was prepared by adding one drop of a 10 per cent commercial wetting agent solution to 10 ml. of 0.1 per cent aqueous solution of amaranth.

Limonene:

The standard used in this investigation was a terpene fraction of oil of

orange, apparently limonene of high purity. The terpene fraction is said to amount to 90–95 per cent or more of cold-pressed citrus peel oils, and is apparently the main objectionable constituent in juices containing excess peel oil. The use of this fraction permits a check on the absolute recoveries obtained in the various types of apparatus. The volatile oil was added to distilled water instead of using a citrus juice.

Procedure:

In all types of apparatus used in these experiments except No. III-mi, measured amounts of 1.00, 0.80, 0.60, 0.40, 0.20, and 0.10 ml. of limonene

TABLE 1.—*Recoveries of limonene in macro-buret volatile oil apparatus*

MODEL NO.	LIMONENE ADDED, ML						AVERAGE LOSSES
	1.00	0.80	0.60	0.40	0.20	0.10	
	ml	*ml*	*ml*	*ml*	*ml*	*ml*	*ml*
I-1	—	—	*	0.33	0.15	0.05	0.06
I-2	0.93	0.77	0.55	0.35	0.13	0.04	0.06
I-2-a	0.93	0.71	0.55	0.35	0.13	0.04	0.07
I-3	0.92	0.74	0.52	0.31	0.12	0.04	0.08
II	0.97	0.76	0.56	0.38	0.18	0.08	0.03
III	0.97	0.76	0.56	0.37	0.16	0.07	0.04
III-w	0.98	0.78	0.58	0.37	0.17	0.08	0.02

* Oil recycled; accurate reading not possible; larger quantities not tried.

TABLE 2.—*Recoveries of limonene in micro-buret volatile oil apparatus (III-mi)*

	ml				
Limonene added	0.250	0.200	0.150	0.100	0.050
Measured directly in buret*	0.262	0.212	0.157	0.101	0.054
Measured after distillation	0.258	0.204	0.151	0.096	0.046
Loss	0.004	0.008	0.006	0.005	0.008

* Average of two or more measurements.

were added separately to one liter of distilled water in a two- or three-liter round-bottom flask. The same 1-ml. graduated pipet was used for all measurements, and all were measured from the zero mark. A small piece of porous plate was added to prevent bumping (this is not necessary with a citrus juice), the apparatus assembled, the oil-recovery trap filled with water, and the flask heated with a Tirrell burner. The reflux or distillation was carried out at a rate of about two drops per second. In Types I and II the reflux period was one hour, in Type III, 200 ml. of distillate was collected. In No. III-mi, measured amounts of 0.250, 0.200, 0.150, 0.100, and 0.050 ml. of limonene were added, 100 ml. of distillate was collected, and

the wetting agent was always used. After the heating was stopped, the apparatus was allowed to drain for several minutes. In Nos. III-w and III-mi-w it was then washed down with a few drops of wetting agent. The oil was then lowered into the buret, and readings made at intervals of two or three minutes until constant values were obtained.

Results:

The results with various types of macro-buret apparatus are given in Table 1 and with the micro-buret in Table 2.

DISCUSSION

Models of Type I.—Table 1 shows that the average recoveries with all models of Type I were significantly lower than in Types II and III. In No. I-1 the oil column divided, returning a part to the distillation flask. An attempt to avoid this difficulty by raising the oil layer above the top of the buret tube, as in Nos. I-2-a and I-3, resulted in even lower average recoveries. The low recoveries in Type I are probably due to several factors. The limonene distills over as a minimum boiling mixture with water, which condenses higher up on the condenser than water alone, and may not be removed completely even by a prolonged period of reflux. Further, it is almost impossible to rinse off the cold-finger condenser with water without forcing some of the oil down through the buret and back to the distillation flask. Also the surface of the oil layer in the trap is in rather close contact with the incoming steam, and some volatilization seems to occur continuously.

Model No. II.—The recoveries with the J. B. Wilson apparatus were about as high as in any of the macro-buret models. The trap below the buret permits the rinsing off of the condenser at the end of the distillation without danger of forcing oil droplets back to the distillation flask; the surface of the oil in the trap is considerably more remote from the incoming vapors and hence is less likely to be revolatilized. However the buret is not as easily cleaned with a brush or by a detergent as in most models. Another disadvantage is the considerably longer time required for the contents of the trap to cool to room temperature before measuring the oil.

Model No. III.—One objection in the citrus industry to the use of apparatus of either Type I or II is that too much time is required for a determination. The usual period of distillation is one hour, and a considerable further period is required to heat the sample to boiling, especially when a two-liter sample is used. In apparatus of Type III, a determination requires a shorter period of time than in the cold-finger condenser-type apparatus since the condenser is continually and completely rinsed down by the condensing vapors. Recoveries of limonene in this apparatus were as good as in any of the others. Though 200 ml. of distillate was usually collected, tests in which from 50 to 200 ml. was collected showed practically

identical recoveries. At the rate of one drop of distillate per second, only about 30 minutes would be required for a 100-ml. distillation. The use of the wetting agent caused the condensers to drain somewhat more rapidly, aided in breaking up emulsions in the trap, and eliminated irregular oil columns. The larger diameter tube below the buret acts as a trap for any oil droplets that might be carried through the buret. The bottom of this tube is fitted with a rubber stopper, making a non-fragile base on which to rest the apparatus on the bench top. Removing the rubber stopper makes the buret more readily accessible for cleaning with a brush. In practice the apparatus is easily cleaned *in situ*. The leveling tube is depressed to drain the contents of the trap, the apparatus is then flushed with water, followed by a detergent such as 5 per cent trisodium phosphate, and finally flushed with water again. This apparatus is the most easily cleaned of any tested. The use of a hemispherical joint in place of the stopcock in No. III-mi makes the leveling tube less fragile.

Model No. III-mi.—Of the three micro-buret types tested the best recoveries of limonene were obtained with the McKinnis apparatus. In the micro-buret apparatus the use of a wetting agent is essential in reading the volume of oil in the buret. When not used, excessive amounts of water adhere to the walls of the buret beside the oil layer, and irregular meniscuses and interfaces, and broken oil columns, are the rule.

When a micro-buret apparatus is used, a smaller sample of juice (500 ml. instead of one liter or more) may be employed, thereby saving considerable time in the distillation. In model No. III-mi the average loss of limonene was 0.006 ml. (Table 2), equivalent to 0.0012 per cent based on a 500-ml. sample of juice. With the corresponding macro-buret model (No. III-w) the average loss was 0.02 ml., equivalent to 0.002 per cent on a one-liter sample of juice, or 0.001 per cent on a two-liter sample. This indicates that results are obtainable with the micro-buret apparatus which are as accurate as with the macro-buret models. The cleanliness of the apparatus is a much more critical factor in the micro-buret apparatus.

Average Losses.—Tables 1 and 2 indicate that the losses of limonene in the several models tested were nearly constant for a given model, and not proportional to the amount of limonene used. It appears that for each model the loss of limonene is nearly the same over a wide range.

In order to obtain more nearly absolute values for volatile oil, it would be advisable to test the apparatus with a series of samples of freshly-distilled limonene or steam-distilled citrus-peel oil in order to determine the average loss, and to add this value to the results obtained. This would obviate a direct calibration of the buret.

SUMMARY

A comparison was made of a number of types of apparatus for the determination of volatile oil in citrus juices. Two types in common use and one new type are discussed in detail.

The lowest recoveries of added limonene were obtained with a commonly-used modification of the Clevenger apparatus. Recoveries with the apparatus of J. B. Wilson were about as high as in any tested. A modified form of the McKinnis model was judged to be the most satisfactory of all, the recoveries of added limonene being as high as in any other apparatus used. Much less time is required for a determination with this apparatus. It is simple in construction and can be easily cleaned.

The McKinnis model, in which a 0.4 ml. micro-buret replaces the 2-ml. macro-buret used in most types of apparatus, was found to be very satisfactory. Recoveries of added limonene were comparable with those obtained with the 2-ml. buret models. A 500-ml. sample of juice may be used so that much less time is required for a distillation. The use of a few drops of a wetting agent to rinse down the condenser at the end of the distillation is essential; this is of benefit also in the corresponding 2-ml. buret model.

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ACKNOWLEDGMENT

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DETERMINATION OF WATER-INSOLUBLE FATTY ACIDS IN CREAM AND BUTTER

By FRED HILLIG (Food Division,* Food and Drug Administration, Federal Security Agency, Washington, D. C.)

There have been numerous studies on the organic acids of cream, butter, and other dairy products, but most workers have been concerned either with the problem of determining a specific acid or of simply titrating the free acids in milk, cream, butterfat, or in the distillates of dairy products.

Turning to butter, Breazeale and Bird (1) have made a comparison between the former A.O.A.C. method (2) for free fatty acids in oils with the method of Clarke *et al.* (3) and with their own modification of the alcoholic

* W. B. White, Chief.

KOH method of Leathes (4), reporting fair agreement among the three. But in the same paper they give a method for isolating, weighing, and titrating the free acids of butterfat. They report that the titer of the isolated acids is less than the titer of the fat itself, and advance the theory that volatile acids in the fat may account for the discrepancy. Not only does their method fail to determine free volatile acids in the fat; it fails to determine all free acids in the water phase and all acids present as their inorganic salts. The method, further, is not applicable to cream.

The investigation reported here was designed to determine *all* the water-insoluble acids (abbreviated hereafter as WIA) in butter or cream, whether present as such or as alkaline salts, and whether in the fat or water phase of the butter. A secondary purpose was to afford a determination of *all* the volatile acids, whether free or as salts. This paper, however, deals only with the WIA, and it will suffice here to say that the volatile acids may be determined by distilling the WIA filtrate and separating the volatile acids by a procedure already published (5).

The method precipitates the WIA in crystalline form, thus separating them from such water-soluble acids as butyric, lactic, and acetic. The mean molecular weight of the WIA so separated indicates that they consist principally of oleic and palmitic acids produced by the partial biological hydrolysis of milk fat.

RECOVERY EXPERIMENTS

Table 1 gives the recoveries of known amounts of oleic and palmitic acids from water solution. The acids* were added as 0.1 *N* alcoholic solu-

TABLE 1.—*Recovery of oleic and palmitic acids by precipitation*

ACIDS PRESENT			ACIDS FOUND		MEAN MOLECULAR WEIGHT	
OLEIC	PALMITIC	TOTAL	TOTAL		FOUND	CALCULATED
mg	*mg*	*mg*	*mg*	*per cent*		
140	128	268	267	99.6	274	270
70	64	134	128	95.5	lost	—
140	64	204	200	98.0	274	274
140	128	268	264	98.5	271	270
281	256	537	530	98.7	272	270
70	256	326	310	95.1	262	262
562	128	690	684	99.1	281	277

tions which were neutralized to phenolphthalein with normal NaOH, diluted with about 10 ml of water and evaporated to dryness on the steam bath. The soaps were then dissolved in 50 ml of water, the acids were precipitated, filtered, weighed, titrated, and the mean molecular weight computed. Recovery and mean molecular weight checked closely with the calculated values.

* C. P. Oleic acid obtained from Eimer and Amend; palmitic acid obtained from Eastman Kodak Company.

TABLE 2.—*Recovery of WIA added to 50 g of cream*

SAMP. NO.	ACIDS ADDED				BLANK ON CREAM		ACIDS FOUND			
								NET RECOVERY		
	OLEIC	PALMITIC	TOTAL	MEAN† MOL. WEIGHT	WEIGHT	MEAN MOL. WEIGHT	TOTAL	WEIGHT	PER CENT	MEAN MOL. WT.
	mg	*mg*	*mg*		*mg*		*mg*	*mg*		
1	—	25.6	25.6	266	28.2	275	55.3	27.1	105.9	263
							52.8	24.6	96.1	259
2	—	128.0	128.0	259	28.2	275	150.3	122.1	95.4	258
							152.0	123.8	96.7	256
3	127.1*	—	127.1	265	55.6	269	185.9	130.3	102.5	266
							182.0	126.4	99.4	267
4	42.4*	25.6	68.0	265	28.2	275	92.1	63.9	94.0	256
							92.3	64.1	94.3	256
5	68.4	64.0	132.4	270	28.2	275	151.9	123.7	93.4	267
							153.1	124.9	94.3	268
6	68.4	124.2	192.6	267	28.2	275	214.4	186.2	96.7	263
							212.4	184.6	95.8	263
7	68.4	248.3	316.7	263	28.2	275	330.4	302.2	95.4	262
							326.0	297.8	94.0	261
8	84.8*	64.0	148.8	262	28.2	275	170.3	142.1	95.5	267
							169.5	141.3	95.0	268
9	136.7	64.0	200.7	274	28.2	275	218.6	190.4	94.9	270
							216.5	188.3	93.8	270
10	205.1	248.3	453.4	268	28.2	275	452.9	424.7	93.7	267
							462.0	433.8	95.7	266
11	418.5	49.7	468.2	278	55.6	269	499.9	444.3	94.9	278
							500.4	444.8	95.0	280
12	418.5	248.3	666.8	272	55.6	269	718.8	663.2	99.5	282
							702.5	646.9	97.0	280
								Av.	96.3	

* Sodium oleate used. Mean molecular weight of precipitated acids, 263.
† Computed from acids in cream and from acids added.

Tables 2 and 3 show the recovery of oleic and palmitic acids added in analogous manner to 50 g of cream and to 50 g of butter, respectively. The WIA were determined by the method presented at the end of this paper.

TABLE 3.—*Recovery of WIA added to 50 g of butter*

SAMP. NO.	ACIDS ADDED				ACIDS FOUND			
	OLEIC	PALMITIC	TOTAL	MEAN† MOL. WEIGHT	TOTAL	NET RECOVERY		
						WEIGHT	PER CENT	MEAN MOL. WEIGHT
	mg	*mg*	*mg*		*mg*	*mg*		
1	42.4*	—	42.4	272	100.6	40.8	96.2	279
					100.9	41.1	96.9	276
2	127.1*	—	127.1	268	188.2	128.4	101.0	265
					190.6	130.8	102.9	268
3	—	25.6	25.6	271	84.4	24.6	96.1	268
					86.9	27.1	105.9	263
4	—	128.0	128.0	263	185.8	126.0	98.4	265
					184.7	124.9	97.6	265
5	—	256.0	256.0	260	313.8	254.0	99.2	264
					315.6	255.8	99.9	264
6	42.4*	25.6	68.0	269	127.7	67.9	99.9	276
					129.7	69.9	102.8	274
7	42.4*	64.0	106.4	266	163.4	103.6	97.4	261
					167.2	107.4	100.9	261
8	68.4	64.0	132.4	272	190.7	130.9	98.9	271
					191.3	131.5	99.3	270
9	68.4	124.2	192.6	268	244.5	184.7	95.9	266
					251.7	191.9	99.6	266
10	136.7	64.0	200.7	275	252.2	192.4	95.9	271
					253.1	193.3	96.3	272
11	205.1	248.3	453.4	269	507.4	447.6	98.7	267
					505.2	445.4	98.2	266
12	55.8	248.3	304.1	263	355.0	295.2	97.1	263
					360.1	300.3	98.8	263
13	418.5	49.7	468.2	279	514.0	454.2	97.0	278
					507.8	448.0	95.7	272
14	418.5	248.3	666.8	273	687.3	627.5	94.1	274
					681.3	621.5	93.2	275

* Sodium oleate used. Mean molecular weight of precipitated acids, 263.
† Computed from acids in butter and from acids added. Blank on butter used was 59.8 mg of WIA of mean molecular weight 278 per 50 g.

In both cases the recoveries are satisfactory and the mean molecular weights closely check the calculated values.

WIA IN COMMERCIAL CREAM AND BUTTER

The method was applied to several commercial samples of cream and butter. The results are given in Tables 4 and 5, respectively.

TABLE 4.—*WIA in commercial cream*

SAMPLE NO.	WIA MG/100 G FAT	MEAN MOLECULAR WEIGHT
1	110	—*
2	122	—*
3	153	—*
4	158	—*
5	166	—*
7	179	—*
8	250	277
9	264	257
10	414	274
11	828	270
12	1060	279
13	1358	281
14	1526	275
15	2022	272

* Difficult to determine accurately at this level.

TABLE 5.—*WIA in commercial butter*

SAMPLE NO.	WIA MG/100 G FAT	MEAN MOLECULAR WEIGHT
1	51	—*
2	66	—*
3	101	—*
4	114	—*
5	143	278
6	238	271
7	278	278
8	414	276
9	505	277
10	621	278
11	964	273
12	1278	281

* Difficult to determine at this level. The results indicate that there are very wide swings in the WIA content of commercial butter and in the cream from which it is made.

DETERMINATION OF WATER-INSOLUBLE ACIDS IN BUTTER

Sampling of butter.—Proceed as directed in *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.107.**

Preparation of butter sample.—Proceed as directed in *Methods of Analysis*, 6th Ed., **22.108** or **22.109.** Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 10 ml of water, if necessary remelt in warm water bath (not steam bath), and add 50 ml of ether. Shake until fat is dissolved.

Preparation of cream sample.—Proceed as directed in *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.60.** Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 20 ml of alcohol, shake, and add 50 ml of ether.

DETERMINATION

Add *N* NaOH in increments of about 0.2 ml to the material in the centrifuge bottle, neutralizing to a decided pink color, using 10 drops of a 1 percent solution of phenolphthalein as indicator, shaking between additions of the alkali until neutralization is complete. Then add 0.5 ml in excess and shake again for at least 2 minutes. During this and all subsequent shakings release the pressure carefully several times to avoid blowing the stopper out and losing some of the contents. (It is difficult to shake more than one bottle at a time because of greasy stoppers and the pressure which develops.) After shaking remove the stopper and add 50 ml of petroleum ether, shake a few times and centrifuge for 5 minutes at about 1200 r.p.m. (longer if separation not sharp). Set the bottle on a horizontal surface and siphon[1] off the ether fat layer.[2] Wash the aqueous layer remaining in the centrifuge bottle by adding 25 ml of ether, mix thoroughly by shaking for several seconds, add 25 ml of petroleum ether and again mix by shaking. Centrifuge, siphon off the ether layer as before, and repeat the washing as above. After each washing the basic red color of phenolphthalein should be permanent; if not, add additional phenolphthalein and alkali to give a decided red (not pink). Add 1 ml of sulfuric acid (1+1) to the residue in the centrifuge bottle and shake vigorously for a few seconds. Add 5 ml of a 10 percent sodium tungstate solution and again shake vigorously a few seconds. After the addition of the sodium tungstate, the material should be distinctly acid to congo red paper; if not, add more of the sulfuric acid. Now add 75 ml of ether, shake violently for at least 2 mintues and centrifuge. (When working with cream, emulsions may form which do not break completely on centrifuging. These can be broken by adding 10 to 20 ml of alcohol, mixing gently and again centrifuging.) Siphon off the ether layer into a 500 ml separatory funnel. Wash the siphon inside and out with 75 ml of ether in such a manner that the washings drain into the centrifuge bottle, shake violently for at least 2 minutes, centrifuge and siphon off the ether layer into the separatory funnel. Slight opalescence of the ether layer may be disregarded. Add 100 ml of dilute alcohol (1+1) to the combined extracts in the separatory funnel, neutralize in same manner as before with *N* NaOH to a decided pink color, add 0.5 ml excess and shake violently for an additional 2 minutes. Immediately add 25 ml of water, mix by single inversion of the funnel, and allow to separate until the water layer is clear. This usually occurs in a few minutes. Slow separation may sometimes be hastened by playing a fine stream of water on the ether surface.

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[1] The siphon is similar to the delivery tube of an ordinary wash bottle except the intake end is bent, in opposite direction to the outlet end, into a U shape, the opening being ¼ to ½ inch higher than the bottom of the U and cut off horizontally. Excessive constriction should be avoided in the bending. The delivery tube is set loosely enough in the stopper so that it can be raised and lowered. In operation it is so adjusted that the opening of the U bend is about ¼ inch above the surface of the aqueous layer. The ether layer can then be blown off gently by means of the customary mouthpiece tube inserted in an adjacent hole in the stopper.

[2] If the ether layer, after centrifuging, is reddish in color, add 10 ml of water, shake and again centrifuge as before. If the reddish color still persists in the ether layer, add 25 ml of ethyl ether, shake and again centrifuge.

If the volume of the emulsion at the interface is only about 10 ml it may be included in the subsequent extraction. Draw off the aqueous layer into a 600 ml beaker. Add 50 ml of the 1 +1 alcohol and about 10 drops of phenolphthalein to the contents of the separatory funnel and neutralize with the alkali, shaking vigorously for about 2 minutes. Add 50 ml of water, mix by single inversion of the funnel and allow to separate until the water layer is clear. Draw off the aqueous layer into the beaker. Add 10 ml of water to the contents of the separatory funnel, mix by single inversion, allow to separate until the water layer is clear, and draw off into the beaker. Place the beaker containing the combined extract and washings on a steam bath (or carefully heat on a hot plate), in order to expel any ether. Evaporate to about 25 ml (a small fan is useful if foaming is serious). (The decided red color should persist through all these operations and up to the point where the soaps are acidified.) Transfer to a 250 ml beaker with about 25 ml of water. (As an alternate procedure the material may be evaporated to dryness on a steam bath and the residue dissolved in about 50 ml of water.) Dissolve 5 g of anhydrous sodium sulfate in the warm solution, using heat if necessary. Cool to room temperature, stirring at frequent intervals in order to keep the soaps from forming a hard crust on the surface. Make acid by adding sulfuric acid (1 +1) dropwise, using congo red paper as indicator. Stir vigorously to affect thorough liberation of the fatty acids, mashing all pink soap curds. Add about 500 mg of a filter aid and mix. Filter with suction into a suitable filter.[3] Rinse the beaker with 3 approximately 15 ml portions of water at room temperature and transfer the rinsings to the crucible. Maintain suction *for several minutes after visible dripping has ceased,* in order to dry the precipitate. Heavy precipitates can be sucked drier if the cracks are plastered up with some of the precipitate. Filtrate should be clear. Substitute a tared beaker[4] or flask, containing a few glass beads or grains of sand, for the receiving flask of the filtering apparatus. Extract the acids with 4 portions (ca 15 ml each) of ether, breaking up the precipitate with a stirring rod between extractions and thoroughly mixing with the ether. The asbestos pad must not be disturbed. Evaporate the ether extract (which should be no more than faintly opalescent) on a steam bath and dry the acids in a 100°C oven for one hour. Cool and weigh. Report results as mg of water-insoluble acids (WIA) per 100 g of butterfat.

Dissolve the weighed acids in 10 ml of neutral benzene, and titrate with 0.1 *N* sodium ethylate, using 10 drops of phenolphthalein as indicator, until the end point holds at least 1 minute. (If desired, neutral alcohol and 0.1 *N* NaOH may be used.) Compute the mean molecular weight of the fatty acids by dividing the mg of acids found by the ml of 0.1 *N* alkali used for the titration, and multiplying by 10. The mean molecular weight should not exceed 290. When the amount of acids is below 150 mg per 100 g of butterfat, the mean molecular weight is without significance.

SUMMARY

A method is proposed for the determination of the water-insoluble acids (including those present as inorganic salts) in cream and butter. The practiced analyst can make approximately 8 determinations per day.

Acknowledgment is made to R. E. Duggan and Dorothy Montgomery of this Administration for their assistance in the development of the method.

[3] The following set-up was used in this work: a bell jar; a Gooch crucible, with removable bottom charged with a thin layer of asbestos overlaid with a small quantity of filter aid. The asbestos was a long fibre, amphibole variety, acid and alkali washed for Gooch crucibles, and washed twice by decantation. The filter aid was Dicalcite Company's "Speedex" added from a suspension in water. Coarse fritted glass crucibles overlaid with a small quantity of filter aid are satisfactory.

[4] Weighed with similar vessel as counterpoise.

REFERENCES

(1) *J. Dairy Sci.*, **21**, 335 (1938).
(2) *Official and Tentative Methods of Analysis, A.O.A.C.*, 4th Ed., 1935, p. 417.
(3) *This Journal*, **20**, 475 (1937).
(4) J. B. Leathes, "The Fats," Longmans, Greene, & Co., London, 1913, p. 74.
(5) *This Journal*, **28**, 644 (1945).

SOME EXAMPLES OF THE OCCURRENCE OF MACHINERY SLIME IN CANNING FACTORIES

By J. D. Wildman[1] and Perry B. Clark[2]

The occurrence of a growth of microorganisms on the exposed surfaces of machinery in tomato factories was noted by Howard.[3] The prominent mold in the slime was identified by Thom as *Oidium lactis*. Howard had found that this mold, when growing on machinery, exhibited a characteristic branching and tapering at the growing ends of the mycelium. Because of the typical appearance of the vegetative mold it was possible to detect machinery mold in the finished tomato product. The present report presents findings of the writers on tomato plants in California, Utah, and Colorado in the summer of 1943.

Machinery slime, when present, may occur on surfaces exposed to juices from the tomatoes, but it is more apt to be found in locations difficult to reach by ordinary cleaning methods, such as complicated conveyor systems, or hoppers not readily accessible for cleaning. The locations where machinery slime was found are given below:

Location	*No. of times encountered*
Conveyer belts	6
Guide rails of conveyor belts	6
Chutes in tables (for peelings, etc.)	5
Inside of hopper below crusher	4
Inside of screw-type conveyor	4
Inside of pans and buckets used in peeling	4
Peeling tables	3
Inside of spray washer hoods	3
Fins of elevator from washer	1
Paddles in washer	1
Sinks used in peeling	1
Inside of pipe from cyclone	1

The machinery slime was found on wood, rubber, composition, and metal surfaces, and in some instances even on smooth, stainless steel. All types of tomato material were found coming in contact with the machinery slime: whole untreated tomatoes, cold trimmed stock, scalded

[1] Division of Microbiology, Food and Drug Administration, Washington, D. C.
[2] San Francisco Station, Food and Drug Administration, San Francisco, Calif.
[3] U.S.D.A. Bull. #569, 1917.

stock, tomato trimmings and peels, peeled tomatoes, crushed tomatoes, and cyclone juice.

To detect slime it was found necessary to inspect all possible areas which might be contaminated. Where the surface being inspected was covered with tomato material it was necessary to wipe away the upper layers of material in order to discover the slime underneath. When freed from tomato material, the slime is usually white to gray in appearance and it often adheres to the supporting surface very tenaciously. When scraped up on a knife blade it has a tendency to form into a pasty lump. As noted by Howard, the presence of machinery slime in a factory is often disclosed by its characteristic odor.

Machinery slime has been found to consist of molds, bacteria, and yeasts, present either singly or in combination. The typical mold *Oidium lactis* found by Howard is readily recognized in the finished product by its type of branching and the pointed condition of the growing ends of the mycelium.

Products from plants in which machinery slime was noted, when examined by the official method for mold,[4] disclosed evidences of machinery mold. It thus became of interest to explore the possibilities of estimating the extent of the contamination by means of a technique which would give the total number of machinery mold filaments per gram of product. The following method was devised for this purpose:

METHOD

Slide preparation: Weigh out 20 g of product and dilute as directed with 1% algin soln, previously prepared by adding the required amount of dry algin to water, mixing in a rapid-acting "blendor," neutralizing to *p*H 7.0–7.5 with NaOH soln, and preserving with 2% of commercial formaldehyde soln. Dilute juice to 40 ml, light puree and light catsup to 60 ml, heavy puree and heavy catsup to 80 ml, and paste to 120 ml, with the algin soln. Mix the diluted material thoroughly and pipette 0.1 ml onto the end of a standard 26×77 mm glass slide. Spread material evenly so as to cover an area, 26×38.5 mm, on one end of slide. Dip this end gently into a 10% soln of calcium chloride until smear has been covered. Remove and dry lower surface of slide by blotting.

Examination: Use magnification of ca 100 times and a field diameter of 1.382 mm. By means of a mechanical stage, move slide so that five strips of material are viewed, each strip extending across the entire width of the slide (26 mm). Locate the five strips at approximately equal distances over the length of the slide covered with the preparation. If slide tends to dry out, dip film again into calcium chloride soln and continue examination. Count the number of separate, characteristic machinery mold fragments occurring in the five strips of material examined and from this calculate the number per g of product. A specimen calculation on juice is given below:

Mold from machinery found in 5 strips examined = 1
Area on which material was spread = 26×38.5 mm = 1001.0 sq mm
Area of 5 strips = 1.382 mm×26 mm×5 = 179.66 sq mm
No. of 5-strip areas per slide = 1001.0 ÷ 179.66 = 5.57

[4] *Methods of Analysis, A.O.A.C.*, 6th Ed., Par. 42.57, p. 788.

TABLE 1.—*Number of machinery molds per g of product*

FACTORY	LOCATION OF MACHINERY SLIME	NO. OF MACHINERY MOLDS PER G OF FINISHED PRODUCT
	Juice	
1	On composition conveyor	111
3	Inside of spray hood	0
6	Washer paddles	0
7	Metal fins of elevator from washer & conveyor rails	111
11	Trimming pans and table	0
	Canned Tomato Packing Medium	
2	Tables, chutes, worm conveyor, crusher box, conveyor	
	(1st spl)	334
	(2nd spl)	334
	(3rd spl)	1,169
8	Grading belt, conveyor rails	
	(1st spl)	666
	(2nd spl)	555
9	Conveyor rails, chutes and crusher box	0
10	Conveyors, chutes	222
12	Crusher box, worm conveyor	222
13	Conveyor rails, worm conveyor, chutes	0
	Puree, Catsup, and Chili Sauce	
2	Peeling tables, chutes, screw conveyor, crusher box, compound conveyor	1,166
5	Peeling tables	0
6	Conveyor rails	0
12	Crusher box, worm conveyor	0
14	Pipe from cyclone	0
15	Pans	0
18	Worm conveyor, conveyor, chutes, spray hood	167
	Paste	
4	Inside of spray hood	0
19	Conveyor rails	0
	Canned Tomatoes	
2	Tables, chutes, worm conveyor, crusher box	
	(1st spl)	0
	(2nd spl)	111
	(3rd spl)	333
8	Grading belt, conveyor rails	111
9	Conveyor rails, chutes	222
10	Conveyors, chutes	0
16	Conveyor rails, sinks used for peeling	111
17	Pans, tables, conveyor	
	(1st spl)	0
	(2nd spl)	333

No. of 5-strip areas per g of orig. material = $5.57 \times 10 \times 2 = 111.4$, in which 2 = dilution factor
No. of machinery molds per g of sample = 111.0.

The factors for converting the number of molds found to the number per gram in the various products are as follows:

Juice $(55.7 \times 2) = 111$
Light purees and catsups $(1+2)(55.7 \times 3) = 167.1$ (use 167)
Heavy purees and catsups $(1+3)(55.7 \times 4) = 222.8$ (use 223)
Paste $(1+5)(55.7 \times 6) = 334.2$ (use 334).

RESULTS

Machinery mold counts by the above method on products made in plants showing machinery slime are shown in Table 1. For comparison, two samples of juice, three of packing medium, and ten of puree and catsup from plants where no slime was noted were examined. All of the samples gave a zero count.

SUMMARY AND CONCLUSIONS

Attention is called to the fact that dirty machinery in tomato plants may still be found. The counts for machinery mold indicate that factory condition may be relatively bad and the count in the finished product may still not be high. On the other hand, the presence of any machinery molds in a product was found to be associated with the presence of machinery mold in the factory.

THE DETERMINATION OF DEXTROSE AND LEVULOSE IN CANE PRODUCTS CONTAINING UNFERMENTABLE REDUCING SUBSTANCES

By F. W. Zerban and Carl Erb (New York Sugar Trade Laboratory, New York, N. Y.)

In the methods devised in this laboratory for the determination of dextrose and levulose in cane sugar products[1,2,3,4] it was tacitly assumed that these two sugars are the only reducing substances present in solutions of the products after clarification with neutral lead acetate. It has been known for many years that cane sugar products, and especially molasses, contain in addition varying quantities of reducing substances which differ from dextrose and levulose in not being fermented by baker's yeast. Zerban and Sattler[5] have recently called attention to the importance of this fact in the analysis of sugar products and in sugar factory control.

In the present investigation an attempt has been made to correct the dextrose and levulose, found in cane molasses by the methods now in use, for the unfermentable reducing substances. In a mixture of two pure

[1] Zerban and Wiley, *Ind. Eng. Chem., Anal. Ed.*, **6**, 354 (1934).
[2] Zerban, *Ind. Eng. Chem., Anal. Ed.*, **8**, 321 (1936).
[3] Erb and Zerban, *Ind. Eng. Chem., Anal. Ed.*, **10**, 246 (1938).
[4] Zerban, *Sugar*, **34**, No. 5, 43 (1939).
[5] Zerban and Sattler, *Sugar*, **42**, No. 2, 44 (1947).

reducing sugars each can be determined by the combination of any two reduction methods, provided that the reducing ratios of the sugars are sufficiently different to afford a reliable basis for the calculations.[6] Similarly, a mixture of three pure reducing sugars can be analyzed by combining three reduction methods that fulfill the same condition. Zerban and Sattler[7] have shown that in a mixture of dextrose, levulose, and maltose each of the three can be determined satisfactorily by combining the method of Jackson and Mathews[8] for the selective determination of levulose, the Zerban-Sattler modification of the Steinhoff copper acetate method[7] for the determination of mono-saccharides in the presence of reducing disaccharides, and Steinhoff's method for the determination of total reducing sugars by means of Fehling solution.[9] When only dextrose and levulose are present, each of these can be determined by combining any two of these three methods.

The three equations by the combination of which the single sugars are calculated, in the absence of maltose, are

$$R_1 = 0.0806\ G + F \quad (1)$$
$$R_2 = a\ G + F \quad (2)$$
$$R_3 = G + b\ F. \quad (3)$$

When maltose is present, the third equation changes to

$$R_3 = G + b\ F + c\ M. \quad (4)$$

In these equations

R_1 is mg. apparent levulose by the Jackson and Mathews method
R_2 is mg. dextrose plus levulose, expressed as levulose, by the Steinhoff acetate reagent
R_3 is mg. total reducing sugars, expressed as dextrose, by the Steinhoff method with Fehling solution
G is mg. dextrose
F is mg. levulose
M is mg. maltose
a is the reducing ratio of dextrose to levulose
b and c are the reducing ratios of levulose and maltose, respectively, to dextrose

Dextrose and levulose are calculated as follows:

Equations (1) and (2) combined, $G = (R_2 - R_1)/(a - 0.0806)$
$F = R_2 - a\ G$
Equations (2) and (3) combined, $G = (R_3 - b\ R_2)/(1 - a\ b)$
$F = R_2 - a\ G$
Equations (1) and (3) combined, $G = (R_3 - b\ R_1)/(1 - 0.0806\ b)$
$F = R_1 - 0.0806\ G$

If equations (1), (2), and (4) are combined, dextrose, levulose, and any

[6] Browne and Zerban, "Physical and Chemical Methods of Sugar Analysis," 3rd ed. (1941), pp. 970–976.
[7] Zerban and Sattler, *Ind. Eng. Chem., Anal. Ed.*, **10**, 669 (1938).
[8] Jackson and Mathews, *Bur. Standards J. Research*, **8**, 403 (1932).
[9] Steinhoff, *Z. Spiritusind.*, **56**, 64 (1933).

maltose or its equivalent are calculated as described by Zerban and Sattler.[7]

These methods of determination and calculation have been applied to a Puerto Rican blackstrap and to a refiner's sirup, before and after fermentation with Fleischmann's baker's yeast. The fermentations were carried out according to the directions of the Java Sugar Experiment Station.[10] Although the unfermentable portion of the two products does not contain dextrose or levulose, it does reduce not only Fehling solution, but also the Jackson and Mathews reagent as well as the Steinhoff copper acetate reagent. However, in order to avoid constant repetition of the word "apparent," the reducing substances that act analytically like dextrose, levulose, or maltose are thus simply designated as the sugars in the tables and the following discussion.

The results of the analyses are shown in Tables 1 and 2.

DISCUSSION

The total reducing sugars, determined directly by the Steinhoff method with Fehling solution (R_3), and expressed as invert sugar, were found to be 19.73 per cent in the blackstrap before fermentation, and 5.65 per cent after fermentation. In the refiner's sirup there was 25.46 per cent before, and 3.96 per cent after fermentation. The sum of dextrose and levulose, calculated from the combination of any two methods, must agree with these figures within narrow limits, because the reducing ratio of levulose to invert sugar is about 0.96, and that of dextrose to invert sugar about 1.04, both close to unity. If, for instance, the reducing sugars in the original blackstrap consisted entirely of dextrose, the percentage would be 18.94, and if they consisted entirely of levulose, it would be 20.60. The sum of dextrose and levulose must vary within these two limits.

The sum of dextrose and levulose found by combining R_1 and R_2 is in every case considerably lower than the invert sugar figures given above, and the results are obviously erroneous.

The values obtained for the sum of dextrose and levulose by combining R_2 and R_3 are quite close to the invert sugar values, but in three out of the total four cases the levulose figure is negative, and the percentage ratio of dextrose to total reducing sugars exceeds 100, which is, of course, impossible. This combination therefore, also gives erroneous results.

The combination of R_1 and R_3 gives values for total reducing closely checking those for the invert sugar found directly, and in addition both dextrose and levulose figures are positive in every case. When R_1, R_2, and R_3 are combined, and the "maltose" found, but not actually present, is converted into equivalent dextrose, the dextrose and levulose results are in close agreement with those obtained by combining R_1 and R_3 only. This makes it very probable that these values are correct. This conclusion

[10] "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed. (1931), p. 365.

TABLE 1.—*Analyses of blackstrap*

	BEFORE FERMENTATION, 300 MG. OF MOLASSES TAKEN	AFTER FERMENTATION, 960 MG. OF ORIGINAL MOLASSES TAKEN. CORRECTED FOR VOLUME OCCUPIED BY YEAST
R_1	37.4 mg.	29.6 mg.
R_2	42.6 mg.	32.2 mg.
R_3	57.3 mg.	48.8 mg.

Calculated Results, Per Cent on Original Molasses

	1 DEXTROSE	2 LEVULOSE	3 MALTOSE	4 TOTAL REDUCING SUGARS	5 PERCENTAGE RATIO OF 1 TO 4
R_1 and R_2 combined:					
Original molasses	3.40	12.20		15.60	21.8
Unfermentable residue	0.43	3.05		3.48	12.4
Fermentable sugars	2.97	9.15		12.12	24.5
R_2 and R_3 combined:					
Original molasses	13.73	5.93		19.66	69.8
Unfermentable residue	5.54	−0.50		5.04	109.9
Fermentable sugars	8.19	6.43		14.62	56.0
R_1 and R_3 combined:					
Original molasses	8.40	11.80		20.20	41.6
Unfermentable residue	2.45	2.89		5.34	45.9
Fermentable sugars	5.95	8.91		14.86	40.0
R_1, R_2, and R_3 combined:					
Original molasses	3.73	11.83	8.67	24.23	15.4
Unfermentable residue	0.56	2.91	3.48	6.95	8.1
Fermentable sugars	3.17	8.92	5.19	17.28	18.3
R_1, R_2, and R_3 combined: Maltose expressed as equivalent dextrose—					
Original molasses	8.36	11.83		20.19	41.4
Unfermentable residue	2.42	2.91		5.33	45.4
Fermentable sugars	5.94	8.92		14.86	40.0

has been confirmed by an independent method, devised by Browne.[11]

[11] Browne and Zerban, "Physical and Chemical Methods of Sugar Analysis," 3rd ed. (1941), pp. 991 and 992.

TABLE 2.—*Analyses of refiner's sirup*

	BEFORE FERMENTATION, 260 MG. OF SIRUP TAKEN	AFTER FERMENTATION, 960 MG. OF ORIGINAL SIRUP TAKEN. CORRECTED FOR VOLUME OCCUPIED BY YEAST
R_1	32.7 mg.	23.4 mg.
R_2	37.1 mg.	24.0 mg.
R_3	64.0 mg.	36.9 mg.

Calculated Results, Per Cent on Original Sirup

	1 DEXTROSE	2 LEVULOSE	3 MALTOSE	4 TOTAL REDUCING SUGARS	5 PERCENTAGE RATIO OF 1 TO 4
R_1 and R_2 combined:					
Original sirup	3.00	12.35		15.35	19.5
Unfermentable residue	0.10	2.42		2.52	4.0
Fermentable sugars	2.90	9.93		12.83	22.6
R_2 and R_3 combined:					
Original sirup	27.89	−3.65		24.24	115.1
Unfermentable residue	5.93	−2.26		3.67	161.6
Fermentable sugars	21.96	−1.39		20.57	106.8
R_1 and R_3 combined:					
Original sirup	14.38	11.42		25.80	55.7
Unfermentable residue	1.74	2.28		4.02	43.3
Fermentable sugars	12.64	9.14		21.78	58.0
R_1, R_2, and R_3 combined:					
Original sirup	3.70	11.50	19.40	34.60	10.7
Unfermentable residue	0.01	2.43	2.98	5.42	0.2
Fermentable sugars	3.69	9.07	16.42	29.18	12.6
R_1, R_2, and R_3 combined: Maltose expressed as equivalent dextrose—					
Original sirup	14.14	11.50		25.64	55.1
Unfermentable residue	1.61	2.43		4.04	39.9
Fermentable sugars	12.53	9.07		21.60	58.0

Here the dextrose and levulose are calculated from the direct polarization, invert polarization, and reducing power. This method, applied to the original blackstrap, gave 8.32 per cent dextrose, 11.52 per cent levulose,

19.84 per cent total reducing sugars, in good agreement with the values found by combining R_1 and R_3. The percentage ratio of dextrose to total reducing sugars was 41.9, also checking with the 41.6 found from R_1 and R_3. Browne's method could not be applied to the refiner's sirup, for lack of material. But it is known[12] that in these sirups the dextrose always exceeds the levulose. Furthermore, the typical analysis of refiner's sirup, quoted by Meade,[13] gives a percentage ratio of 56.8, against the 55.7 found in the original sirup by combining R_1 and R_3.

Correction for the unfermentable reducing substances, for the same combination, does not appreciably alter the percentage ratios found in the original products. In the blackstrap the percentage ratio of fermentable dextrose to total fermentable sugars is 40.0, compared to 41.6 in the original material, and in the refiner's sirup the corresponding values are 58.0 and 55.7.

It may be concluded that the actual dextrose and levulose present in cane products can be found by determining the reducing power with the Jackson and Mathews reagent and with Fehling solution as described, before and after fermentation of the product, calculating the percentages of each sugar as shown, and deducting the values found after, from those found before, fermentation. It must be emphasized, however, that there are still several sources of error in the method. In the first place, no correction has been applied for the reducing power of the sucrose contained in the products. This error is small, however, as may be seen from the fact that the Munson and Walker method for the determination of invert sugar in the presence of sucrose gave practically the same results for total invert sugar in the original products as the Steinhoff copper tartrate method without correction for the reducing effect of the sucrose, namely 19.69 per cent in the blackstrap, and 25.65 per cent in the refiner's sirup. Reducing substances that were not present in the original product are known to be formed by fermentation with yeast, and this constitutes another important source of error. The magnitude of this error is being investigated at present in this laboratory, and the results will be published later.

It is evident that the Steinhoff copper acetate reagent, which is being used successfully for the determination of dextrose in starch conversion products, cannot be used for differentiating between monosaccharides and higher saccharides in mixed corn and cane products when the latter contain unfermentable reducing substances, as is usually the case. The analysis of such products is a problem that has not been solved satisfactorily so far.

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[12] Browne, *Louisiana Planter*, **61**, 202 (1918).
[13] Spencer-Meade, "Cane Sugar Handbook," 8th ed. (1945), p. 343.

BOOK REVIEWS

The National Formulary, Eighth Edition. By Committee on the National Formulary, Justin L. Powers, *Chairman.* American Pharmaceutical Association, Washington (1946). 6×9 inches, 888 pages. Cloth.

This new edition of the National Formulary became official on April 1, 1947. It is the second edition published since the U. S. Food, Drug, and Cosmetic Act recognized the N. F. as an "official compendium." It contains 190 more pages than the N. F. VII; 81 of these pages represent an increase in the space devoted to monographs, and 58 are due to expansion of the section on "General Tests, Processes and Apparatus." One hundred and eighty-four new monographs have been added, but 176 monographs that appeared in the N. F. VII have been deleted, so there is a net gain of only 8.

The most striking change is the use of the English instead of the Latin names as the primary titles of the monographs. This has resulted in shifting the positions in the book of many of the monographs, but the change should make the locating of a particular preparation easier for those chemists who do not readily think in pharmaceutical Latin. The repetition of the titles at the edges of the pages adds to the ease of use of the book.

This book will be a necessary part of the library of every official charged with enforcing the drug section of the U. S. Food, Drug, and Cosmetic Act or similar State laws. It is also, however, a repository of a large number of methods for analyzing drugs, and as such it will be valuable to all drug chemists. Among new methods of assay that did not appear in previous editions the following were noted: a formol titration for aminoacetic acid in the elixir; a method for iodochlorohydroxyquinoline in tablets, based on a determination of iodine; an assay for pentobarbital in its elixir; methods for phenylmercuric chloride and nitrate; and a periodate oxidation method for propylene glycol. The method for bismuth in bismuth subcarbonate tablets has been changed so that the bismuth is now precipitated and weighed as the phosphate instead of being precipitated as the basic carbonate and ignited to the oxide. The new monograph on sherry wine sets a limit of 350 p.p.m. for sulphur dioxide and includes an assay for this compound.

A table of optical crystallographic constants for the crystalline drugs that appear in the N. F. VIII should be valuable to chemists who are experienced in crystallographic techniques.

It was noted that the book includes a monograph for Phenolated Calamine Lotion, but no monograph for Calamine Lotion itself, although the directions for making the phenolated lotion give Calamine Lotion as one of the ingredients.

The committee is to be congratulated on both the form and the contents of this new edition.

H. J. FISHER

The Pharmacopoeia of the United States of America, Thirteenth Revision. By Committee of Revision, E. Fullerton Cook, *Chairman.* Mack Printing Co., Easton, Pa. (1947). 6×9 inches, 1064 pages, cloth. Price $8.50.

This edition (U. S. P. XIII) became official on April 1, 1947. It contains 96 more pages than the U. S. P. XII; there are 61 more pages devoted to monographs; an increase of 21 pages in the section on "General Tests, Processes and Apparatus"; and 16 extra pages of Reagents, Test Solutions, etc. Ninety-five monographs have been added and 56 deleted—a net increase of 39. The deletions include such old favorites as whiskey, brandy, ergot and its fluidextract, spirit of camphor, and sulfapyridine. Among drugs appearing for the first time are Bayer 205 (as "suramin

sodium"), dichlorophenarsine and oxophenarsine hydrochlorides, penicillin and its preparations, sodium lauryl sulfate, sulfamerazine, thiopental sodium and Zephiran (as "benzalkonium chloride").

This edition of the U. S. P. differs from all of its predecessors in that the English names are made the primary titles. This has resulted in rearranging the positions in the book of many of the drugs. The typographical arrangement, with the monograph titles repeated at the sides of the pages, is identical with that of the N. F. VIII. Average doses are given in large bold face type. The general monographs on emulsions, extracts, fluidextracts, injections, suppositories, tinctures, triturations, and waters have been transferred to the section on General Tests, Processes, and Apparatus. A table of refractive indices of U. S. P. crystalline substances and a section on testing parenteral solutions for clarity have been added.

Among the points of interest to chemists and enforcement officials the following were noted:

While the bioassay by the cat method is retained for the "assay" of digitoxin, its injection and tablets, two colorimetric methods of assay are described as "colorimetric controls" in the section on "General Tests."

Ergot and its fluidextract have been transferred to the N. F. VIII, where no method of standardization is provided, but a colorimetric method of assay, using the Smith-Allport-Cocking reagent, is prescribed for ergonovine maleate, its injection and tablets.

The title "Tincture of Iodine" has been transferred to the product known in the U. S. P. XII as "Mild Tincture of Iodine," and "Tincture of Iodine U. S. P. XII" has become "Strong Iodine Tincture N. F. VIII." Whatever the therapeutic reasons for this change, it should serve to intensify the confusion that has been shown to exist among retail pharmacists as to what tincture of iodine is.

The diazotization procedure using an outside indicator has been retained for the assay of all sulfa drugs. In the opinion of this reviewer the method of Conway [*J. Am. Pharm. Assoc.*, **34**, 236 (1945)] is to be preferred.

Arsenic is determined in dichlorophenarsine and oxophenarsine hydrochlorides by a bromate titration; in other arsenicals the iodine-thiosulfate procedure is used.

The fluorometric method of assay is prescribed for riboflavin itself, but this method is recognized only as a "control" for riboflavin injection and riboflavin tablets, which must be "assayed" microbiologically.

Methods for iodine in calcium iodobehenate, chiniofon and iodopyracet injection involve the titration of iodide with silver nitrate using starch as an adsorption indicator.

The test for sesame oil is the old Baudouin procedure; the present A.O.A.C. modified Villavecchia test is superior in that the false positive tests obtained with some olive oils by the Baudouin method are eliminated.

An assay is provided for Carbachol (carbamyl choline) that depends on the precipitation of choline as the reineckate and the colorimetric estimation of this salt in acetone solution.

"Benzalkonium chloride" (Zephiran) and its solution are assayed by the ferricyanide method.

A method of assay is provided for dihydromorphinone in its tablets.

The monograph for stearyl alcohol includes a method for "hydroxyl value" that employs acetyl chloride as the acetylating agent.

New standards and methods of assay have been provided for totaquine, its capsules and tablets, but these changes do not become effective until April 1, 1948.

The new U. S. P. is a credit to the committee and the large number of consultants that contributed to its preparation.

H. J. FISHER