FIRST DAY

MONDAY—MORNING SESSION

REPORT ON QUALITY FACTORS IN PROCESSED VEGETABLES

By B. M. GUTTERMAN, R. D. LOVEJOY,* and L. M. BEACHAM (Food and Drug Administration, Federal Security Agency, Washington, D.C.)

PRELIMINARY REPORT ON CORRELATION OF ACETALDEHYDE WITH OFF-FLAVOR IN FROZEN VEGETABLES

For years those interested in the frozen vegetable industry have recognized that inadequately blanched vegetables have subsequently developed progressive "off" flavors, odors, and colors, owing to continued enzyme activity. When present in slight degree these may result only in lowering the quality, but as they increase they reach a point where the product becomes inedible. With this problem in mind the Canned Food Branch of the Division of Food of the Food and Drug Administration was assigned the task of locating one or more methods of objective analysis, readily reproducible, which would provide a workable index to the "off" flavors.

Arighi, Joslyn, and Marsh[1] stated that acetaldehyde serves rather well as an index of off-flavor, etc., resulting from inadequate blanching in the preparation of frozen vegetables. Accordingly this lead was followed in our own investigations. After analysis of several hundred packages of frozen peas and asparagus it has been quite clearly demonstrated that whenever the acetaldehyde level exceeds a certain point the organoleptic analysts have invariably classed the product as being of inferior quality, or inedible.

It is readily apparent that acetaldehyde is not the cause of the off-flavor but appears to be one of the by-products of the reaction producing the unidentified compound or compounds which give the product the off-flavor.

The samples of frozen vegetables were prepared for this study, in cooperation with the frozen food industry, in such a way that the products were processed from field to storage in accordance with good commercial practice, except for variations in blanching to permit a study of that factor. Basically the samples were grouped in sets of three and sometimes four.

1. No blanch
2. Short blanch (one which did not completely inactivate catalase)
3. Normal factory blanch
4. Double normal factory blanch.

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* Associate Referee.

[1] Arighi, A. L., Joselyn, M. A., and Marsh, G. L., *Ind. Eng. Chem.*, 28, 595 (1936)

Fifty-one samples reflecting controlled variation of blanch time were prepared, together with 90 samples of normal factory packs collected from different parts of the country. Several packages of each of the above samples were examined for flavor and acetaldehyde content.

The method of analysis used is a modification of Arighi's method and that given in *Methods of Analysis, A.O.A.C.*, 6th Edition, **16.16**, as a volumetric method for aldehydes in distilled liquors. In outline it is as follows:

Steam distil the product; add excess of bisulfite to distillate; oxidize excess bisulfite w/excess iodine; and back titrate w/thiosulfate.

In our laboratory the method showed good reproducibility. At present two universities are making further studies of the method in connection with frozen peas and frozen asparagus.

We are at present considering the best means of distributing samples for collaborative study. This poses quite a problem, inasmuch as homogeneity of sample is rather difficult to obtain when dealing with products such as asparagus. In addition, the product must be shipped in the frozen state. One plan at the moment is to select samples known to have a zero acetaldehyde value and, after comminuting a large amount of the product, add a known amount of acetaldehyde and freeze.

REPORT ON COLORING MATTERS IN FOODS

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

Following the recommendation of Committee B of the A.O.A.C. the Referee undertook the problem of a quantitative separation and estimation of Sunset Yellow F.C.F. (FD&C Yellow No. 6) from Amaranth (FD&C Red No. 2). The method as published herewith should be considered in the nature of a preliminary report, because a number of minor details may have to be altered.

The procedure is based on the fissure of the dye molecules under definite conditions and combining the first component with a suitable phenolic group to form dye compounds which are more amenable to separation.

METHOD

Prepare a 1% soln of the dye or dye mixture.

For the purpose of orientation it is essential to ascertain whether the sample is either FD&C Red No. 2 or FD&C Yellow No. 6, or a mixture of both dyes. Therefore, dilute a portion of the 1% soln sufficiently to obtain one of 0.005% strength. Measure out or pipette into 2 test tubes, 10 ml each, of this soln. Into each add 0.4 ml of saturated bromine water, mix, followed by 0.2 ml of saturated aqueous hydrazine sulfate, mix, lastly add 0.2 ml of strong ammonium hydroxide. The presence of FD&C Red No. 2 manifests itself by the formation of a deep blue color, if all or almost all consists of Amaranth; purple, if the product is a mixture of both dyes; or a faint pink (rather fleeting) if the amount of FD&C No. 2 is 0.5% or less of the total. The presence of FD&C Yellow No. 6 may be suspected if the re-

sulting coloration is of a bright lemon yellow solution. The intensity of this color will naturally vary as the quantity differs. As a check for FD&C Yellow No. 6, withdraw again into 2 test tubes 10 ml each of the 0.005% soln and add in order named, shaking after each addition: pyridine, 0.4 ml; saturated bromine water, 0.4 ml; saturated aqueous hydrazine sulfate, 0.2 ml; and lastly, strong ammonium hydroxide, 0.2 ml. The formation of a deep orange coloration indicates the presence of FD&C Yellow No. 6. FD&C Red No. 2 treated similarly will give a light yellow soln. The quantity of FD&C Yellow No. 6 appears to be proportional to the depth of the orange coloration. The minimum amount detectable is 0.5%. Therefore, a deep blue coloration by the first test and a light yellow by the second test indicates the presence of FD&C Red No. 2 only; while conversely, a deep orange color by the second test and a lemon yellow soln by the first test indicate that FD&C Yellow No. 6 is the only dye present.

Withdraw several 20-ml portions of the 1% soln and titrate with 0.1 *N* $TiCl_3$ using sodium citrate as buffer, following the procedure as outlined in 6th Ed., **21.39.** Note titer. If the qualitative tests listed above gave evidence of presence of both dyes (FD&C Red No. 2 and FD&C Yellow No. 6) pipette a 20-ml portion of the 1% dye soln into a 100-ml beaker and add 10 ml of (1+1) hydrochloric acid and 40 ml of water. To this soln add gradually in small portions sodium hydrosulfite ($Na_2S_2O_4$) while stirring vigorously until dyes are reduced and soln becomes colorless. Place on steam bath and evaporate to dryness. (It is recommended to run this text in duplicate.) To the dried precipitate in the beaker add 75 ml of hot water, 5 ml of neutral lead acetate (25%), and sufficient strong ammonium hydroxide (0.5–1.0 ml) to produce slight alkalinity. Permit soln to cool and settle, filter and wash with cold water to make 100 ml. To this soln add 10 ml of hydrochloric acid, and 0.2 ml of 10% copper sulfate soln ($CuSO_45H_2O$). Cool contents of flask to 5°C. and filter off precipitate, if present, and add slowly 1 ml of 10% sodium nitrite soln. Keep temp. at 0–5°C. for 2 hours, testing with starch iodide paper at intervals of ca. 30 min. Into a liter beaker measure out 10 ml of a 1% soln of beta naphthol (in dilute alcohol), 100 ml of a 10% soln of anhydrous sodium carbonate, and 40 ml of water. Cool this to ca 15°C. Into the beta naphthol soln pour the diazo soln in small portions, stirring vigorously. Rinse flask with several 20-ml portions of water and add to the beaker. Place beaker over steam bath and maintain a temp. of 70°C. for about 1 hour. Permit cooling, to reach room temp. When cool add 35 ml of glacial acetic acid. Proceed as directed in *Methods of Analysis*, 6th Ed., **21.67**, page 299, fourth line from bottom "Use six separators of 250 ml capacity" and follow method to bottom of page 300. The number of ml of standard $TiCl_3$ required to reduce the Orange II corresponds to the quantity of Sunset Yellow FCF originally present. However, since a recovery of 93.8% was obtained, the factor 1.066 is used to multiply the number of ml of $TiCl_3$ for the corrected value for FD&C Yellow No. 6. Subtract the corrected titration from total color titer. Difference is the volume necessary to reduce FD&C Red No. 2 originally present.

1 ml 0.1 *N* $TiCl_3$ = FD&C Yellow No. 6 = 0.011305 g
1 ml 0.1 *N* $TiCl_3$ = FD&C Red No. 2 = 0.01511 g

RECOMMENDATIONS*

It is recommended—

(1) That additional study be devoted to the technique of the quantitative separation and estimation of FD&C Yellow No. 6 in presence of FD&C Red No. 2 and, if possible, collaborative studies be undertaken.

* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 47 (1951).

(2) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2, FD&C Green No. 3, and FD&C Blue No. 1.

(3) That investigational work be undertaken to separate quantitatively FD&C Yellow No. 3, FD&C Yellow No. 4, FD&C Orange No. 2, and FD&C Red No. 32.

(4) That collaborative work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

REPORT ON OILS, FATS, AND WAXES

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

There will be no reports on spectroscopic methods of oil analysis and on quantitative methods for peanut oil. The Associate Referee on antioxidants has developed a quantitative method for propyl gallate and plans to submit this method to collaborative study during the coming year. At the same time other recently proposed methods for some of the antioxidants will be subjected to this critical study. The Associate Referee on coal-tar colors in oils has reported collaborative work on a simplified and improved method. The results obtained indicate that most of the difficulties inherent in the present official method are avoided in this modified version.

RECOMMENDATIONS*

It is recommended—

(1) That studies on quantitative methods for peanut oil be continued.

(2) That studies on spectroscopic methods for the analysis of oils be continued.

(3) That collaborative work on various methods for antioxidants in oils be conducted by the Associate Referee.

(4) That the present method for coal-tar colors in oils (**26.51, 26.52**), be deleted and that the method proposed by the Associate Referee be accepted as first action.

(5) That further collaborative work on the modified method for coal-tar colors be conducted.

* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 50 (1951).

COAL-TAR COLORS IN OIL

By MARIE L. OFFUTT (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

Some work on the detection of coal-tar colors in oil was started last year and showed the necessity of modifying the present A.O.A.C. method. The work was continued this year and the modified method and three samples of oils with colors added was sent to collaborators. These modifications are essentially refinements in technique and the fundamental procedures of separation and identification of the present method (**26.52**) are followed.

Sample 1 contained Yellow AB and OB (FD&C Yellow Nos. 3 and 4); Quinizarine Green SS (D&C Green No. 6), with a small amount of chlorophyll added. Sample 2 contained Oil Orange SS (FD&C Orange No. 2). Sample 3 contained Oil Orange SS (FD&C Orange No. 2), and Quinizarine Green SS (D&C Green No. 6).

Details of the method used have been published in *This Journal*, **34**, 76 (1951).

COLLABORATOR	SAMPLE 1	SAMPLE 2	SAMPLE 3
1, 2, 3, 4	Yellow AB & OB (FD & C Yellow Nos. 3 & 4) Quinizarine Green SS (D & C Green No. 6)	Oil Orange SS (FD & C Orange No. 2)	Oil Orange SS (FD & C Orange No. 2) Quinizarine Green SS (D & C Green No. 6)

The results of collaborators as reported showed good agreement and the collaborators commented that most of the difficulties of the old method had been removed.

The Associate Referee wishes to express her grateful appreciation to G. Kirsten, who helped in modifying the method, and to the following chemists, all of the Food and Drug Administration, who collaborated in this work: D. W. McLaren, Buffalo District; Leonora Auerbach, New York District; F. J. Sabatino, Philadelphia District; and Helen T. Hyde, San Francisco District.

It is recommended* that the present A.O.A.C. method be deleted and that the modified method be accepted as first action.

* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 51 (1951).

REPORT ON ANTIOXIDANTS

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

Work is in active progress on a method for estimation of propyl gallate in fats and oils. The method involves extraction with 40% alcohol and development of a purple color by the addition of ferrous tartrate solution. The intensity of the color is then determined spectrophotometrically and compared to a curve prepared from known concentrations of the antioxidant. Since the color produced obeys Beer's law in the desired region, excellent accuracy and reproducibility are attainable. Also, the method seems to be selective for propyl gallate among the more common antioxidants.

A private communication to the Referee states that Mahon and Chapman, of the Canadian Department of National Health and Welfare, have devised a series of methods for four commonly used antioxidants. This paper is to be published shortly in another journal. Bentz and Throckmorton, of the Tennessee Eastman Corporation, have also communicated to the author a method for the detection of phenolic antioxidants.

The three methods are now being subjected to critical evaluation by the Associate Referee, and it is hoped to have a unified method ready for collaborative testing during the coming year.

It is recommended* that further work on this subject be continued.

REPORT ON DAIRY PRODUCTS

BY WILLIAM HORWITZ (Food and Drug Administration, Federal Security Agency, Minneapolis 1, Minnesota), *Referee*

Ash and acidity of milk.—The Associate Referee has recommended a method for titratable acidity based upon his experimental studies and collaborative work which fixes the dilution and indicator concentration. He also recommends changing the sample size in the ash method for milk from 10 grams to 5 grams for consistency between the milk and the evaporated milk methods. A statistical analysis of the data by the Referee confirms the conclusion that the precision attained by the collaborators with the smaller size sample does not differ significantly from that attained with the 10 gram sample. There is, however, a significant difference in the results from the different laboratories which indicates that the details of the method are not sufficiently defined. Committee C has recommended a complete review of the moisture and ash methods in the Dairy chapter with a view toward their unification. Such a review may result in a more precise statement of the experimental conditions for these determinations.

* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 50 (1951).

Added water, serum methods.—The last several reports on the serum tests for the detection of added water in milk have demonstrated the inadequacy of the present limits of the refractometer readings to detect anything but the most extravagantly adulterated milks. In general, however, as pointed out by the Associate Referee, the lowering of the refractometer reading follows the quantity of water added and that if the original reading of the milk is known, the quantity of added water may be calculated with a fair degree of accuracy. This suggests that the difficulty in applying the serum tests arises from using a lower limit based upon the most extreme value ever reported for a normal milk. The serum methods appear to be one of the few cases in the *Book of Methods* which include an interpretation of data and general application of the limits specified results in such limited applicability that the method is unjustly discredited as being useless. The refractometer readings vary considerably with such variables as breed of cow, fat content, feed, etc. A possible solution to the difficulty may be to shift the burden of interpreting the results from the *Book of Methods* to each individual laboratory. Each laboratory could accumulate a set of authentic data which would be characteristic of the type of milk normally encountered, and whose refractometer readings may lie considerably above those currently used which are based upon the entire literature on the subject. Even closer limits may be obtained for individual milks by the use of ordinary inspectional techniques designed to obtain authentic samples of the suspected milk. It is therefore suggested that the serum methods be modified by deleting references to specific refractometer readings and ash values, in the official acetic serum method (**15.28**) and the official copper serum method (**15.29**).

Lactic acid.—Partition chromatography has been applied by the Associate Referee to the determination of lactic acid in milk. As would be expected with any such fundamental departure from the normal techniques, the variations encountered were too large to warrant the use of this method by chemists without previous experience. Further work will undoubtedly result in reducing the variations to a reasonable range.

Preparation of butter samples.—Collaborative studies have been performed for the first time using a mechanical shaking machine as an optional substitute for hand shaking for the preparation of butter samples. The Referee concurs with the suggested redrafting of the procedure for the preparation of butter samples by shaking which will now allow the use of a shaking machine. Further work is contemplated by the Associate Referee.

Fat in dairy products.—Work in the Referee's laboratory with ethers containing 0.001 to 0.004 g/100 ml. active oxygen[1] failed to confirm the results of Muers and House[2] that ether-containing peroxides yield high

[1] Siggia, "*Quantitative Organic Analysis via Functional Groups*," Wiley, 1949, p. 100.
[2] Meurs and House, *Analyst*, 74, 85 (1949).

results in fat determinations. The peroxide content of this ether, however, was undoubtedly considerably less than that used by Meurs and House. After work was completed a bottle of very old ether of unknown origin was unintentionally used in an evaporated milk analysis. The results were very high and erratic. Repetition of the analysis with new ether (practically all of the original material having been used in the analysis) gave the expected results:

Can:	*1*	*2*	*3*	*4*	*5*	*6*
%Fat, peroxide ether	9.10	8.49	10.01	9.24	8.67	10.36
%Fat, peroxide-free ether	7.90	7.91	7.92	7.89	7.86	7.92

The ACS peroxide test performed on a few drops of original ether resulted in an intense positive peroxide reaction. This ether was not available for a quantitative active oxygen determination. In view of these results it is recommended that "peroxide-free" be inserted before the word "ether" wherever it appears in the fat methods for dairy products. It is also recommended that the ACS test for peroxides be used rather than the USP test, since the former requires only one minute of standing, whereas the latter requires one hour. The peroxide-containing ether which was used in the first part of this investigation gave an immediate but weak reaction with potassium iodide solution, but the amount of peroxide present was not sufficient to affect the fat results.

Fat, Babcock method.—A. H. Robertson has called attention to a possible misinterpretation of the description of the Babcock pipet in that the words "Nozzle, straight" have been construed as meaning "No constriction at the tip," whereas the intention was to insure delivery parallel to the axis of the pipet and not to the side. The two lines in paragraph **15.26 (b)** "Nozzle, straight" and "Delivery, 5–8 seconds," should be corrected editorially to: "Nozzle parallel with axis of pipet, but slightly constricted so as to discharge in 5 to 8 seconds when filled with water."

Total solids, sweetened condensed milk.—In editing the sixth edition of the *Book of Methods*, changes recommended in the total solids methods for milk and evaporated milk only were also made in the corresponding method for sweetened condensed milk. The correct method as it appeared in the fifth edition was restored in the seventh edition.

RECOMMENDATIONS*

It is recommended—

(1) That method **15.4** (p. 227), acidity of milk, be revised according to suggestion of the Associate Referee and adopted as first action.

(2) That the size of sample in the method for ash in milk be changed from "ca 10 grams" to "ca 5 grams" and that the method be adopted as official.

(3) That the acetic serum method for added water, **15.28 (a)**, be

* For report of Subcommittee C and action of the Association, see *This Journal*, **34**, 48 (1951).

amended by deleting the sentences "Reading below 39 indicates added water; between 39 and 40, addition of water is suspected." and "When reading is 40 or below, determine ash in serum as directed under **(b).**"

(4) That the method for acetic serum, ash, **15.28 (b),** be amended by deleting the sentence "Result below 0.715 g/100 ml indicates added water."

(5) That the method **15.29** for copper serum be amended by deleting the sentences: "Reading below 36 indicates added water." and "When refractometer reading is 36 or below, determine ash of acetic serum as directed under **15.28 (b).**"

(6) That method **15.106** for the preparation of butter samples be revised as suggested by the Associate Referee.

(7) That the Roese-Gottlieb Method for fat (**15.25**) be revised by addition of a footnote to include a specification for the ether used: "*Ether.*—Use ether that passes the following test: To 10 ml ether in a small, clean glass-stoppered cylinder previously rinsed with a portion of the ether under test, add 1 ml of a freshly prepared 10% solution of KI. Shake and allow to stand 1 minute. No yellow color should be observable in either layer." Further, the other fat methods in the Dairy chapter should be revised by substituting the phrase "peroxide-free ether (**15.25**)" for the word ether.

(8) That the description of the Babcock pipet, **15.26 (b)** be revised in accordance with suggestions of the Associate Referee.

(9) That the following subjects be continued:

(a) Preparation of butter samples.
(b) The Babcock test with particular reference to homogenized milk and chocolate drinks.
(c) Sampling hard and soft cheeses.
(d) Reconstituted milk.
(e) Frozen desserts.
(f) Phosphatase test.

REPORT ON ASH AND ACIDITY OF MILK AND EVAPORATED MILK

By G. G. FRARY (State Chemist, Univ. Chemistry Bldg., Vermillion, S. Dak.), *Associate Referee*

Further study of methods for determination of ash in milk and evaporated milk, and of methods for determination of the acidity of milk, was recommended at the 1949 meeting of the Association. The methods are found in Chapter 22, paragraphs **22.4, 22.16** and **22.76,** of the sixth edition of *Methods of Analysis.* In 1939 the Associate Referee and Burton Jordan reported[1] results of a study of methods for total solids

[1] *This Journal,* **23,** 453 (1940).

and ash in milk. Eight collaborators took part in the study and their results showed that direct ashing of the sample, after removal of most of the moisture on the steam bath and without addition of nitric acid (formerly required), gave satisfactory results. Confirmation of these results was sought in the study of this year. Use of a smaller sample than recommended in the 6th Ed. method for ash in milk (**22.16**) seemed es-

TABLE 1.—*Results using present dilution*

(One volume (17.6 ml) of the prepared sample was diluted with an equal volume of CO_2-free water. One-half ml of 1% phenolphthalein in neutral alcohol was used for each titration.)

ANALYST	Ml. 0.1 *N* NaOH			pH AT END POINT			ACIDITY AS LACTIC ACID, %		
	Sample A	*Sample B*	*Fresh Milk*	*Sample A*	*Sample B*	*Fresh Milk*	*Sample A*	*Sample B*	*Fresh Milk*
R.J.B.	2.51	2.50	2.82[a]	8.38	8.38	8.28	0.315	0.313	0.141
	2.52	2.51	2.80	8.38	8.40	8.28	0.315	0.315	0.140
S.H.P.	2.48	2.49	2.64[b]	8.50	8.50	8.51	0.317	0.318	0.132
	2.48	2.49	2.65	8.56	8.53	8.52	0.318	0.318	0.132
W.A.B.	2.47	2.53	2.55[b]	8.52	8.51	8.51	0.316	0.323	0.128
	2.43	2.44	2.59	8.49	8.47	8.54	0.311	0.312	0.129
A.M.O.	2.55	2.55	3.05[c]	8.30	8.29	8.25	0.320	0.320	0.152
	2.50	2.55	3.10	8.39	8.38	8.45	0.313	0.320	0.155
			2.88[d]			8.19			0.144
			2.87			8.22			0.144
E.H.Z.	2.50	2.50	2.90[c]	8.40	8.28	8.25	0.313	0.313	0.145
	2.50	2.48	2.90	8.45	8.37	8.36	0.313	0.310	0.145
			2.90[d]			8.23			0.145
			2.85			8.27			0.143

[a] Pasteurized milk.
[b] Homogenized milk.
[c] Raw Milk No. 1.
[d] Raw Milk No. 2.
Samples A and B were evaporated milk and the values shown for acidity are for the milk as received.

pecially desirable. As pointed out in the previous report, there appears to be no valid reason why the same method may not be used for ash in milk and in evaporated milk. Therefore, in order to have uniform samples, evaporated milk was used, with a request that determinations also be made by each collaborator on fresh whole milk obtained locally.

In 1947 a study[2] of the determination of titrable acidity in milk was reported by Zilliox, Mitchell, and the Associate Referee in which the influence of dilution of sample and concentration of phenolphthalein

[2] *This Journal*, 30, 130 (1947).

indicator were considered. It was shown that in order to obtain consistent results the dilution of the sample and the concentration of the indicator must be uniform and in fixed relation to each other. It also was shown that the "neutral" point could be reached with phenolphthalein at *p*H 8.0–8.3 by diluting the milk sample with twice its volume of water and using more indicator than called for in the method in common use. This year the study of the method by collaborators was undertaken to confirm these results.

TABLE 2.—*Results using more dilution and more indicator*

(One volume (17.6 ml) of the prepared sample was diluted with two volumes of CO_2-free water. One ml of 2% phenolphthalein in neutral alcohol was used in each titration.)

ANALYST	Ml. 0.1 *N* NaOH			*p*H AT END POINT			ACIDITY AS LACTIC ACID, %		
	Sample A	*Sample B*	*Fresh Milk*	*Sample A*	*Sample B*	*Fresh Milk*	*Sample A*	*Sample B*	*Fresh Milk*
R.J.B.	2.30	2.27	2.44[a]	8.14	8.13	7.93	0.288	0.285	0.122
	2.29	2.23	2.46	8.12	8.13	7.92	0.288	0.280	0.123
S.H.P.	2.18	2.18	2.10[b]	8.25	8.20	8.20	0.278	0.278	0.105
	2.17	2.17	2.08	8.22	8.22	8.20	0.278	0.277	0.104
W.A.B.	2.23	2.20	2.20[b]	8.31	8.21	8.22	0.285	0.280	0.110
	2.18	2.16	2.18	8.27	8.21	8.24	0.279	0.276	0.109
A.M.O.	2.25	2.30	2.35[c]	8.28	8.21	8.14	0.283	0.288	0.118
	2.25	2.25	2.35	8.20	8.22	8.12	0.283	0.283	0.118
			2.25[d]			8.11			0.113
			2.26			8.01			0.113
E.H.Z.	2.25	2.20	2.25[c]	8.09	8.15	8.05	0.283	0.275	0.113
	2.25	2.25	2.28	8.15	8.07	8.08	0.283	0.283	0.114
			2.30[d]			8.05			0.115
			2.45			8.17			0.123

Note—Identity of samples same as Table 1.

Instructions to collaborators were to prepare the evaporated milk sample as directed in paragraph **22.73**, *Methods of Analysis*, 6th Ed., and determine acidity in this prepared sample by the tentative method, paragraph **22.4**. The acidity was to be found also by diluting the sample, measured by use of the Babcock 17.6 ml pipette, with twice its volume of CO_2-free water and using 1 ml of 2% phenolphthalein. In each case the titration was to be carried to the first definite pink color and the *p*H was to be read immediately upon completion of the titration.

The instructions as to ash determination were simply to compare results

obtained by using 5 gm and 10 gm portions of the prepared samples and follow the tentative method, **22.16**, in order to determine whether the smaller portion of sample could safely be used without lessening the accuracy of the method.

Table 1 gives the results obtained by collaborators when using the dilution and amount of indicator called for in the present tentative method for acidity, **22.4**. Table 2 gives the results obtained by further dilution of the milk sample and the use of more indicator.

Results above tabulated confirm the findings of earlier work (*loc. cit.*) that dilution of the milk sample and use of more indicator enable earlier detection of an end point while at the same time less alkali is used. Also, the *p*H of the neutral solution is lower and in the desired range between 8.0 and 8.3.

TABLE 3.—*Ash in milk, % by weight*

Comparison of results, using 5 and 10 grams sample weights

ANALYST	SAMPLE A		SAMPLE B		FRESH MILK	
	10 grams	*5 grams*	*10 grams*	*5 grams*	*10 grams*	*5 grams*
R.J.B.	1.555	1.555	1.405	1.372	0.735[a]	0.732
	1.535	1.540	1.385	1.382	0.725	0.734
S.H.P.	1.500	1.492	1.451	1.459	0.709[b]	0.711
	1.504	1.505	1.443	1.437	0.714	0.712
W.A.B.	1.507	1.513	1.470	1.439	0.712[b]	0.699
	1.513	1.505	1.456	1.457	0.708	0.705
A.M.O.	1.560	1.560	1.511	1.505	0.766[c]	0.770
	1.554	1.565	1.499	1.525	0.759	0.767
					0.769[d]	0.764
					0.758	0.768
E.H.Z.	1.536	1.540	1.493	1.497	0.769[c]	0.769
	1.541	1.540	1.488	1.508	0.769	0.771
					0.767[d]	0.764
					0.767	0.768

See Table 1 for footnotes. The samples used were the same as those used for acidity determination and identified in Table 1.

The results of ash determinations made on the same samples with 5 and 10 gram portions, respectively, show that equally satisfactory results may be obtained with the smaller weight of milk. This supports the official method for ash in evaporated milk, **22.76**, wherein 4 to 5 grams of the prepared sample are used. Complete ashing is easier and more quickly attained by use of the smaller weight of sample. Two collaborators sug-

gested that wetting down the ash, drying and re-igniting might be an improvement, but if the weight of sample be limited to 5 grams this modification is felt to be unnecessary.

Thanks of the Associate Referee are due the collaborators and are hereby extended to R. J. Baker, South Dakota State College; Sam H. Perlmutter and William A. Bosin, Minneapolis Station of the Food and Drug Administration; and Arlene M. O'Rourke and E. H. Zilliox of the South Dakota State Chemical Laboratory.

RECOMMENDATIONS*

It is recommended the method for acidity of milk, **22.4,** be amended to read as follows and as so amended be adopted as official:

Measure or weigh a suitable quantity (ca 20 ml or 20 g) of the sample into a suitable dish and dilute with twice its volume of CO_2-free water. Add 2 ml of 1% phenolphthalein soln, **39.31 (d),** and titrate with 0.1 *N* NaOH to the first persistent pink shade. If a measured volume of sample was used determine its weight from the specific gravity of the sample. Report acidity as per cent of lactic acid by weight. If the Babcock milk pipette, **15.26 (b),** be used, the number of ml of 0.1 *N* NaOH required ÷ 20 = per cent acid as lactic acid.

This wording makes no fundamental change in the method. It simply makes definite the amount of sample to be used, where the present wording only suggests this amount, and it modifies the extent of dilution and the quantity of indicator to be used.

It is also recommended that the method for ash in milk be amended by changing the amount of sample to be taken for the determination from "ca 10" grams to "ca 5" grams, and that the method so amended be adopted as official.

REPORT ON THE PREPARATION OF BUTTER SAMPLES

By ALBERT L. WEBER (Food and Drug Administration, Federal Security Agency, New York, New York), *Associate Referee*

At the 63rd meeting of the A.O.A.C., the following pertinent recommendation was adopted:

That methods **22.108** and **22.109,** preparation of butter samples, and the tentative method given in *This Journal,* **31,** 91 (1948) be adopted as "procedures" and that the Referee and Associate Referee make diligent effort to devise one or more methods, whether or not involving the use of mechanical shakers, stirrers, etc., that will be satisfactory to analysts.

In line with the above recommendation, further work was done and several analysts were asked to collaborate. The following instructions were sent to the collaborators:

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* For report of Subcommittee C and action of the Association, see *This Journal,* **34,** 48 (1951).

INSTRUCTIONS TO COLLABORATORS

Collection of Samples

Collect five consecutive one pound prints of butter as they come from cutter of printing machine. Cut prints into quarters, place opposite quarters in a regular or wide mouth Mason jar fitted with a flat metal disc with a rubber-like gasket. Cover secured by a threaded ring. Label jars IA and IB. Treat the other 4 prints likwise and label jars IIA and IIB to VA and VB.

Methods

Method I. Suggested redraft of Method **22.108.**

Soften entire sample in a closed Mason jar (fitted with a flat metal disc with a rubber-like gasket cover secured by a threaded ring) by warming in a water bath maintained at about 40°C., shaking intermittently to reincorporate any separated fat and to observe fluidity of sample as softening progresses. When optimum fluidity is obtained, shake vigorously (by hand at room temperature) until homogeneous semi-solid mass is obtained. Weigh the portion for analysis without undue delay. If prepared sample is kept longer than ½ hour before a portion is weighed for analysis, again soften and reprepare as directed above before withdrawing portions for analysis.

Method II. Follow directions of Method I, with these exceptions:

A. Melt butter in water bath maintained at about 50°C., shaking frequently while melting to prevent breaking of emulsion.

B. Shake under running cool water (water temperature not to be less than 15°C. nor greater than 22°C.).

Method III. The Meuron Method as described in *This Journal,* **29,** 126 (1946).

Method IV. Same as Method I, but jars to be shaken in a bottle-shaking machine at moderate speed.

Method V. Follow directions of Method IV, with these exceptions:

A. Melt butter in water bath maintained at about 50°C, shaking frequently while melting to prevent breaking of emulsion.

B. Shake like Method IV with fan blowing on the jars.

Have the jar number correspond to the method number, *e.g.,* Jars IA and IB should be analyzed by Method I, etc.

In Methods I, III, and IV, withdraw two portions for analysis, one from the upper half of the jar and the other from the lower half of the jar. To get the lower half portion, either spoon off the upper half first or withdraw the portion with a glass tube. A tube about 9 inches long and 0.4 inch in diameter is satisfactory. In Methods II and V, withdraw two portions for analysis, one from the middle portion of the jar and one from next to the glass. To get the second portion, a spoon can be used. Report curd and salt as well as moisture determinations. Please give room temperatures at time of analysis.

All comments and criticisms would be appreciated.

Table 1 gives the results of the reporting collaborators.

TABLE 1.—*The moisture, curd and salt, and butterfat (by difference) obtained by different methods of preparation of the butter*

METHOD	SUB	MOISTURE—PER CENT							
		G.E.K.[1] 24°C.[2]	G.E.K. 26.5°C.	M.L.D. 23.5°C. TO 24.0°C.	M.L.D. 29°C. TO 31°C.	H.O.M. 22°C.	A.L.W. 22.5°C.	A.L.W. 28.0°C.	H.J.M. 21.5°C. TO 25.0°C.
I	A—Top	16.40	16.57	16.35	15.87	16.57	16.32	16.20	16.38
	Bottom	16.37	16.61	16.33	15.95	16.63	16.37	16.22	16.41
	B—Top	16.39	16.56	16.31	15.96	16.63	16.42	16.24	16.29
	Bottom	16.41	16.56	16.36	15.98	16.63	16.42	16.20	16.28
III	A—Top	16.42	16.52	16.43	15.94	16.26	16.28	16.20	16.10
	Bottom	16.41	16.52	16.28	15.89	16.27	16.30	16.18	16.13
	B—Top	16.42	16.53	16.35	15.96	16.39	16.35	16.24	16.01
	Bottom	16.44	16.51	16.40	15.89	16.45	16.39	16.26	16.04
IV	A—Top	16.36	16.57	16.35	15.93	16.73	16.26	16.28	16.66
	Bottom	16.34	16.57	16.32	15.92	16.91	16.21	16.26	16.57
	B—Top	16.38	16.62	16.31	15.89	16.57	16.25	16.20	16.48
	Bottom	16.35	16.62	16.36	15.89	16.70	16.27	16.26	16.60
II	A—Middle	16.46	16.56	16.36	16.01	16.82	16.49	16.27	16.60
	Sides	16.44	16.29	16.39	16.01	16.84	16.38	16.18	16.68
	B—Middle	16.54	16.45	16.41	15.93	16.72	16.46	16.24	16.18
	Sides	16.42	16.31	16.37	15.97	16.81	16.40	16.18	16.45
V	A—Middle	16.55	16.62	16.26	15.96	16.69	16.42	16.28	16.63
	Sides	16.57	16.56	16.29	15.83	Liquid	16.40	16.20	16.61
	B—Middle	16.43	16.56	16.35	15.89	16.69	16.38	16.24	16.65
	Sides	16.47	16.52	16.34	15.90	Liquid	16.42	16.20	16.63

[1] Collaborator.
[2] Room temperature when analysis was made.

METHOD	SUB	SALT AND CURD—PER CENT							
		G.E.K. 24°C.	G.E.K. 26.5°C.	M.L.D. 23.5°C. TO 24.0°C.	M.L.D. 29°C. TO 31°C.	H.O.M. 22°C.	A.L.W. 22.5°C.	A.L.W. 28.0°C.	H.J.M. 21.5°C. TO 25.0°C.
I	A—Top	2.91	3.17	3.51	3.81	2.74	2.75	2.64	3.06
	Bottom	2.91	3.15	3.48	3.85	2.66	2.75	2.60	2.99
	B—Top	2.90	3.15	3.50	3.84	2.66	2.73	2.60	2.97
	Bottom	2.85	3.14	3.49	3.90	2.71	2.70	2.62	2.96
III	A—Top	2.93	3.16	3.28	3.81	2.65	2.69	2.68	3.04
	Bottom	2.91	3.11	3.29	3.79	2.54	2.63	2.68	3.02
	B—Top	2.90	3.17	3.41	3.77	2.59	2.66	2.66	3.03
	Bottom	2.94	3.15	3.47	3.80	2.64	2.62	2.60	3.07
IV	A—Top	2.96	3.16	3.50	3.87	2.71	2.70	2.64	3.06
	Bottom	2.91	3.17	3.49	3.89	2.73	2.73	2.60	2.95
	B—Top	2.90	3.18	3.47	3.87	2.75	2.71	2.60	2.96
	Bottom	2.95	3.14	3.49	3.83	2.74	2.68	2.64	2.98
II	A—Middle	2.89	3.16	3.92	3.71	2.74	2.74	2.68	3.00
	Sides	2.91	3.07	3.95	3.73	2.67	2.69	2.65	2.96
	B—Middle	2.90	3.09	3.77	3.79	2.66	2.70	2.66	2.95
	Sides	2.92	3.07	3.83	3.77	2.67	2.65	2.66	3.02
V	A—Middle	2.95	3.17	3.42	3.85	2.70	2.74	2.62	2.86
	Sides	2.94	3.11	3.47	3.91	Liquid	2.72	2.66	2.93
	B—Middle	2.89	3.15	3.55	3.93	2.67	2.73	2.68	2.97
	Sides	2.91	3.06	3.39	3.90	Liquid	2.70	2.64	3.06

TABLE 1 (*continued*)

METHOD	SUB	BUTTERFAT—PER CENT							
		G.E.K. 24:C.	G.E.K. 26.5°C.	M.L.D. 23.5°C. TO 24.0°C.	M.L.D. 29°C. TO 31°C.	H.O.M. 22°C.	A.L.W. 22.5°C.	A.L.W. 28.0°C.	H.J.M. 21.5°C. TO 25.0°C.
I	A—Top	80.69	80.26	80.14	80.32	80.69	80.93	81.16	80.56
	Bottom	80.72	80.24	80.19	80.20	80.71	80.88	81.18	80.60
	B—Top	80.71	80.29	80.19	80.20	80.71	80.85	81.16	80.74
	Bottom	80.74	80.30	80.15	80.12	80.66	80.88	81.18	80.76
III	A—Top	80.65	80.32	80.29	80.25	81.09	81.03	81.12	80.86
	Bottom	80.68	80.37	80.43	80.32	81.19	81.07	81.14	80.85
	B—Top	80.68	80.33	80.24	80.27	81.02	80.99	81.10	80.96
	Bottom	80.62	80.34	80.13	80.31	80.91	80.99	81.14	80.89
IV	A—Top	80.68	80.27	80.15	80.20	80.56	81.04	81.08	80.28
	Bottom	80.75	80.26	80.19	80.19	80.36	81.06	81.04	80.48
	B—Top	80.72	80.20	80.22	80.24	80.68	81.04	81.20	80.56
	Bottom	80.70	80.24	80.15	80.28	80.56	81.05	81.10	80.42
II	A—Middle	80.65	80.28	79.72	80.28	80.44	80.77	81.05	80.40
	Sides	80.65	80.64	79.66	80.26	80.49	80.93	81.17	80.36
	B—Middle	80.56	80.46	79.82	80.28	80.62	80.84	81.10	80.87
	Sides	80.66	80.62	79.80	80.26	80.52	80.95	81.16	80.53
V	A—Middle	80.50	80.21	80.32	80.19	80.61	80.84	81.10	80.51
	Sides	80.49	80.33	80.24	80.26	—	80.88	81.14	80.46
	B—Middle	80.68	80.29	80.10	80.18	80.64	80.89	81.08	80.38
	Sides	80.62	80.42	80.27	80.20	—	80.88	81.16	80.31

COMMENTS OF COLLABORATORS

George E. Keppel, Minneapolis District, U. S. Food and Drug Administration:

We now use Method IV for practically all of our regulatory work. Before we obtained our shaking machine last year from your district, we used a method very similar to Method I, except that after opening the jar the butter was stirred vigorously with a long-handled spoon for a few seconds before withdrawing a portion with the same spoon for analysis. We use uncovered dishes for our butter work.

I use the Meuron method (Method III) occasionally and prefer it in the case of samples that have had a chance to come to room temperature before analysis.

In connection with this method, I noticed a phenomenon that I missed previously, and which might have some significance in connection with this problem. With the Meuron method (stirring at room temperature) the salt and curd particles are quite coarse and flaky, as compared to the fine particles obtained by the other methods in which the same butter was heated to 40 or 50°C. Presumably the coarse particles would be easier to wash free from fat, and would be less likely to pass through the filter. This might make it possible to use a filter that is more convenient than the Gooch crucibles now used.

Matthew L. Dow, St. Louis District, U. S. Food and Drug Administration:

None of the methods requiring softening at 50°C. were satisfactory because of the complications of separation of the fat and curd. No amount of shaking seemed to prevent this separation. The most convenient method was IV, in which the shaking machine relieved the Analyst of the labor of shaking the jars while the butter cooled. The Meuron method is very rapid and convenient, but I wonder if further shaking isn't necessary to prevent separation after mixing, at least until the sample is no longer fluid. A combination of the Meuron and machine-shaking methods might be the best of all the methods.

When the room temperature during analysis was between 29°C. and 31°C., the butter was rather fluid (except Sample No. II) after preparation and each jar was shaken thoroughly by hand immediately before portions were withdrawn for analysis. The butter prepared by machine shaking became an air emulsion which appeared to be stable.

Harry O. Moraw, Chicago District, U. S. Food and Drug Administration:

The conditioning of butter samples to the semi-solid state after softening at 40° to 50° centigrade is not practicable because it is too time-consuming. It required about 1½ hours on the two samples softened at 40°, and one hour at 50° cooled by hand shaking in cold water. One sample softened at 50° cooled by a fan in a mechanical shaker was still in a liquid state after five hours of shaking.

On the basis of the results I have obtained in the past on both original and check samples on numerous butter samples, which were conditioned to a thick viscous liquid and then shaken and stirred nearly up to the time of weighing, I am convinced that it is unnecessary for samples to be in a semi-solid state. Consideration should be given to replacing the words "semi-solid" to "thick viscous liquid" and the provision included for shaking and/or stirring up to, or just before, weighing the sample.

A thick fluid conditioning can be attained quickly, or under practical working conditions, with multiple samples, whereas the semi-solid requirement would multiply the work-time per sample many times. Overnight tempering should be done at all times where practicable."

Your Referee has used Method IV for some time and finds it most satisfactory. When the room temperatures are above 25°C., he does not allow the butter to become as fluid as when the room temperatures are below 25°C. Under these conditions, it generally takes 20 to 30 minutes shaking to get the butter in a semi-solid state. Once in a while a fan has to be used. When the butter can come to room temperature, without the use of water, and only a few samples are to be analyzed, I find Method III convenient.

I have also noted the particle size of the curd and salt when Method III is used and expect to do further work on this point.

Your Referee had the collaborators use two jars of butter for each method rather than duplicate aliquots from one jar, for he considered this a check on the method and in a way, a check on the Analyst.

The results reported in Table 1 show that Methods I, III, and IV are quite satisfactory.

When the butter is softened in a water bath whose temperature is around 50°C., cooling aids are needed during shaking and erroneous results might be obtained, especially when the jar is held under cool running water.

Consideration will be given to collaborator Moraw's comments regarding the change from "semi-solid" to "thick viscous liquid."

RECOMMENDATIONS*

It is recommended—

(1) That paragraphs **22.109** and **22.110** be deleted.

(2) That paragraph **22.108** be revised to read as suggested in redraft.

(3) That recommendation (2) become first action.

(4) That the Meuron Method as described in *This Journal,* **29,** 126 (1946), be made first action.

(5) That further collaborative work be done, particularly on Methods III and IV.

No report was given on fat in dairy products.

* For report of Subcommittee C and action of the Association, see *This Journal,* **34,** 48 (1951)

REPORT ON DETECTION OF ADDED WATER BY THE SERUM TESTS

By H. J. HOFFMANN (Department of Agriculture, Dairy and Food, St. Paul, Minnesota), *Associate Referee*

In April 1946 the Association of Food, Feed, and Drug Officials of the South Central States in their convention requested the A.O.A.C. to make a study of the Sour Serum refractometric test for added water in milk, the idea being to devise a quantitative procedure and re-investigate the qualitative limits of this test. A review of the history of the serum methods and a study of some results were reported at the 1947 convention of the A.O.A.C. and published in *This Journal*, **31**, 124, 1948, with the recommendation "that the study of the serum methods for added water in milk, *i.e.*, Acetic Serum, Sour Serum, and Copper Serum, be continued." This recommendation was carried out during the year 1948 and the results of this study were published in *This Journal*, **32** (1949), on pages 309–317.

On the basis of these studies the Associate Referee made certain recommendations to the Association as follows:

(1) Further study of the serum methods with the objective of making the test more effective in screening out suspected samples.

(2) Abandon efforts to make any of the serum methods quantitative.

(3) Make the present official copper and acetic serum methods tentative until sufficient new data is available.

(4) Drop the present sour serum method, since evidence shows that it could not be made into a quantitative procedure and because of the difficulty of performing this determination since modern market milk sours with difficulty.

Furthermore, the Committee C made the recommendation that the Associate Referee conduct fundamental studies of the acetic serum method and also the copper serum method and that the sour serum method be dropped, first action.

Although further study on the acetic and copper serum methods was not accomplished in 1950, a study of the figures submitted by the collaborators on this problem in 1948 was made.*

In going through the tables published with that study it appeared that by rearranging them, further light might be thrown on the whole situation. With this in view, Tables 1 to 5, inclusive, and Tables 9 to 13, inclusive, were arranged in such a manner as to show the immersion refractometer readings of the different unwatered milks in conjunction with those readings of the watered sample. The rearrangement of these tables follows:

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* See *This Journal*, **32**, 309 (1949).

Sour serum method

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN % OF ADDED WATER (SOUR SERUM) REFRACTOMETER READINGS 20° C.				
	Pure	*5%*	*10%*	*15%*	*20%*
A. Holstein Herd (15 cows)	42.5	41.1	39.6	37.6	36.2
B. Predominately Brown Swiss, some Holsteins (10 cows)	43.7	42.1	40.5	39.1	37.5
C. Holstein Herd (8 cows)	41.4	39.7	37.7	36.7	35.1
D. Mixed Herd (7 cows)	41.4	39.8	38.2	36.9	35.4
E. Mixed Herd (12 cows)	42.7	40.9	39.7	38.1	37.1
Guernsey Herd	43.3	41.2	39.8	38.0	36.6
Market Milk Grade A	40.2	38.6	37.3	36.0	34.6
Market Milk Grade A	41.2	39.6	38.0	36.6	35.2
Refractometer reading at 20° C. Average Difference from unwatered milk, Average	42.0	40.3	38.8	37.3	35.9
	0.0	1.7	3.2	4.7	6.1

Acetic serum method

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN % OF ADDED WATER (ACETIC SERUM) REFRACTOMETER READING 20° C.				
	Pure	*5%*	*10%*	*15%*	*20%*
A. Holstein Herd (15 cows)	42.0	40.6	39.3	38.1	36.7
B. Predominately Brown Swiss, some Holsteins (10 cows)	43.2	41.7	40.2	38.7	37.2
C. Holstein Herd (8 cows)	41.9	40.5	39.1	37.7	36.4
D. Mixed Herd (7 cows)	42.0	40.6	39.1	37.8	36.4
E. Mixed Herd (12 cows)	42.7	41.3	39.8	38.3	36.8
Guernsey Herd	43.6	42.1	40.8	39.0	37.6
Market Milk Grade A	41.6	40.3	38.7	37.2	35.9
Market Milk Grade A	42.0	40.8	39.3	37.7	36.3
Refractometer reading at 20° C. Average Difference from unwatered milk, Average	42.3	40.9	39.5	38.0	36.6
	0.0	1.4	2.8	4.3	5.7

Copper serum method

DESCRIPTION OR NUMBER OF SAMPLE	MILK CONTAINING KNOWN % OF ADDED WATER (COPPER SERUM) REFRACTOMETER READING 20° C.				
	Pure	*5%*	*10%*	*15%*	*20%*
A. Holstein Herd (15 cows)	38.4	37.4	36.3	35.3	34.3
B. Predominately Brown Swiss, some Holsteins (10 cows)	39.1	38.1	37.0	36.0	35.0
C. Holstein Herd (8 cows)	38.1	37.1	36.3	35.3	34.3
D. Mixed Herd (7 cows)	38.2	37.2	36.2	35.2	34.2
E. Mixed Herd (12 cows)	38.2	37.2	36.3	35.3	34.3
Guernsey Herd	38.6	37.5	36.5	35.5	34.4
Market Milk Grade A	37.3	36.3	35.4	34.3	33.4
Market Milk Grade A	37.6	36.7	35.7	34.8	33.7
Refractometer reading at 20° C. Average Difference from unwatered milk, Average	38.2	37.2	36.2	35.2	34.2
	0.0	1.0	2.0	3.0	4.0

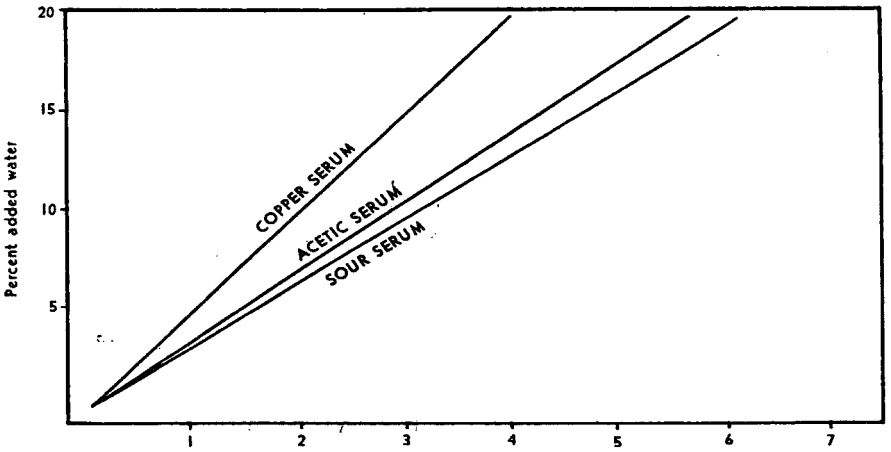

FIG. 1.—Reduction in immersion refractometer reading at 20°C.

If a graph (Fig. 1) is now constructed making use of the sour serum data, the percentage of added water may be plotted as ordinate, while the average of the numerical reduction noted in the refractometer is plotted as abscissa; it will then be noted that an approximately straight line will develop. In like manner graphs are made using data developed from the acetic acid serum method and the copper serum method. The inference to be drawn from these lines is that the reduction in the refractometer readings of the respective serums is directly proportional to the per cent of watering, and that therefore these refractometer readings can be used to determine the per cent of added water in milk.

While this may be true for each individual or herd milk, it has no real practical value to the analyst because of the great variance of refractometer readings of milk serums. If the analyst is to proceed using the minimum refractometer reading of 38.3 (sour serum), 39 (acetic serum), and 36 (copper serum), then without question a considerable percentage of water could be added to milk having a high refractometer reading, which percentage would not be detected.

Since from the graph it appears that the depression registered on the immersion refractometer readings of the respective serums is very nearly a constant figure for a definite percentage of watering, one should be able to predict the maximum per cent of water which could be added to a milk before detection of it is possible by using the minimum serum figure as given in the A.O.A.C. methods of analysis (**22.28, 22.29, 22.30**). To demonstrate this, maximum serum readings of herd milk are given, together with A.O.A.C. minimums, and their differences.

	SOUR SERUM[1]	ACETIC SERUM[2]	COPPER SERUM[3]
Herd Milk	43.9	43.8	39.2
A.O.A.C. Minimum	38.3	39.0	36.0
Difference	5.6	4.8	3.2

[1] *This Journal*, **2**, 150 (1916).
[2] *Ind. Eng. Chem.*, **13**, 198 (1921).
[3] *This Journal*, **31**, 124 (1948).

From the graph it will now be seen on the sour serum line that the point 5.6 intersects at the ordinate point 18.25. In other words, a milk with a sour serum refraction of 43.9 could possibly be watered to the extent of about 18% without falling below the 38.3 minimum. In like manner, the 4.8 difference on the acetic serum line can be interpreted to mean 16.75 on the ordinate or a possible watering of about 16% without falling below the 39 A.O.A.C. minimum. The copper serum difference of 3.2 can also be shown to be equivalent to a possible 16% addition of water without falling below the A.O.A.C. minimum.

SUMMARY

1. Any normal milk having serum refractometer readings above the A.O.A.C. minimums may be subject to watering to a greater or lesser extent.

2. Detection of watered milk using serum tests and A.O.A.C. minimum values is almost impossible except for the grosser adulterations.

3. None of the serum tests can be made to apply quantitatively using the minimum values given in the methods in the interpretation of the results.

4. Any interpretation of serum refractometric data, as regards added water in milk, must be dependent upon the investigation of the serum of milk from the same source in its unaltered normal condition.

CONCLUSION

(1) That the numerical values set up in the methods for the interpretation of results be deleted and include in the methods that the results obtained on an unknown sample are to be compared with serum readings of a sample of milk of known purity from the same source from which the unknown sample was obtained.

(2) From the reduction in the serum readings, if any, between the results obtained on the suspicious sample and the authentic sample, the percentage of added water can be quantitatively estimated from Figure 1, using the proper serum curve. The percentage of added water will be shown as the ordinate if the reduction in the serum reading is plotted as the abscissa.

REPORT ON LACTIC ACID IN DAIRY PRODUCTS (CHROMATOGRAPHIC METHOD)

By LLOYD C. MITCHELL (Food and Drug Administration, Federal Security Agency, Minneapolis, Minnesota), *Associate Referee*

In 1948, H. V. Claborn and W. I. Patterson published a chromatographic method for the determination of lactic and succinic acids in foods.[1] In 1950 F. Hillig modified it somewhat in his work on the determination of succinic acid.[2] The method for lactic acid follows the succinic acid procedure in order that both acids may be determined on the same ether extract if desired. A change, however, was made in the preparation of the silicic acid column in that the ingredients are mixed by shaking them together with the solvent in a separatory funnel, instead of grinding them together with a mortar and pestle before making the slurry with the solvent.

Two samples of nonfat dried milk solids (dried skim milk), and one sample of dried whole eggs were submitted to the collaborators, together with a sample of recrystallized barium lactate to be analyzed for lactic acid.

METHOD

APPARATUS

(*a*) *Continuous extractor.—Methods of Analysis*, 1945, sec. 22.8.
(*b*) *Chromatographic tube.*—ca 17 mm O D×300 mm.
(*c*) *A source of air pressure*, equipped with a pressure regulator.
(*d*) *Lamp*, giving a long narrow band of "cold" light.

REAGENTS

(*a*) *Solvents* (with exception of $CHCl_3$, all solvents should be distilled after alkalizing with NaOH (1+1)):
(1) *20% tertiary butanol* in $CHCl_3$ (v/v).
(2) *Ether.*
(3) *Alcohol.*—95%.
(4) *Alcohol*, dilute—(9+2).

(*b*) *Indicators:*

(1) Glycerol indicator soln.—Ammonium salt of 3,6-disulfo-beta-naphthalene-azo-N-phenyl-alpha-naphthylamine (R-NH_4 indicator, also called Alphamine Red-R). Dissolve 20 mg of dye in ca 10 ml H_2O in a 100 ml volumetric flask, add 60 ml glycerol, 1 drop *N* NH_4OH, mix, make to 100 ml with H_2O, and mix.
(2) *Phenol red indicator.*—0.1% in 20% alcohol.
(3) *Phenolphthalein indicator.*—1% in alcohol.

(*c*) *Acid solns:*

(1) *Acetic acid soln.*—Make 0.5 ml up to 100 ml with $CHCl_3$.
(2) *Phosphotungstic acid.*—20%.
(3) *Sulfuric acid.*—ca Normal.
(4) *Sulfuric acid.*—(1+1).

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[1] *This Journal*, 31, 134 (1948).
[2] *This Journal*, 33, 842 (1950).

(*d*) *Alkali solns:*

(1) *Ammonium hydroxide.*—ca Normal.
(2) *Barium hydroxide.*—Saturated.
(3) *Sodium hydroxide.*—(1+1).

(*e*) *Standard solutions:*

(1) *Barium hydroxide.*—0.01 Normal.
(2) *Zinc sulfate.*—0.01 Normal (by weight).

(*f*) *Chemicals and miscellaneous:*

(1) *Acetone.*
(2) *Barium lactate.*
(3) *Carborundum,* 20 grain (silicon carbide), or glass beads, or porcelain chips.
(4) *Filter aid* (filter cel).
(5) *Filter paper circles* cut with cork borer to loosely fit chromatographic tube.
(6) *Lactic acid.*
(7) *Norite* (charcoal).
(8) *Silicic acid.*

PREPARATION OF PARTITION COLUMN

Place 10 g silicic acid into a dry pear-shape 125 ml separatory funnel, add 30 ml of 20% tertiary butanol-$CHCl_3$ solvent, shake thoroly, add 4.0 ml glycerol indicator soln (the optimum amount of indicator soln may vary with changing H_2O content of the silicic acid and with different lots of silicic acid) and 1 drop *N* NH_4OH; shake vigorously until mixture is homogenous, transfer slurry formed to chromatographic tube which is plugged at constricted end with cotton capped with circle of filter paper and clamped in slight oblique position, spin tube a few times in upright position, and apply pressure (9–10 pounds; *caution:* anchor stopper to prevent it from blowing out) to top of tube forcing excess solvent dropwise out of constricted end, releasing pressure just as liquid disappears at top of gel (otherwise gel cracks and column is useless).

STANDARDIZATION OF COLUMN

(*A*) *Preparation of stock solution.*—Dissolve 0.8757 g barium lactate in H_2O and dilute to 100 ml. Each ml contains 5 mg lactic acid.

(*B*) *Separation technic:*

Transfer 2 ml stock soln to 50 ml beaker, add ca 5 ml alcohol, make alkaline to phenolphthalein with saturated $Ba(OH)_2$, and evaporate to dryness on steam bath. Add 3 drops H_2SO_4 (1+1), rub with stirring rod until the barium salt is thoroly wet, and stir in 200 mg anhydrous Na_2SO_4.

After placing 50 ml graduated cylinder under prepared column, add carefully to preserve the level surface of the gel 1 ml acetic acid soln in $CHCl_3$ to the column. Add 2 ml tertiary butanol-$CHCl_3$ solvent to contents of 50 ml beaker, mix, and immediately decant soln of lactic acid into the prepared partition column, pouring it slowly down side of tube and sink it into gel under pressure. Rinse beaker with three 1 ml portions of tertiary butanol-$CHCl_3$ solvent, decant washings to column, and apply pressure each time until solvent just disappears into gel. After last washing has sunk into gel, wash down sides of column with 2 ml solvent and sink into gel. Fill tube with tertiary butanol-$CHCl_3$ solvent and renew pressure. Collect percolate until the lower edge of the blue band, second from the outlet end, reaches a point 2–5 mm above narrowest portion of constriction of tube (or above filter paper circle). The volume collected is the threshold volume(s) for lactic acid. (The first band to leave column is acetic acid.) Change receiver and collect 35–50 ml percolate.

Transfer percolate to 250 ml glass stopper Erlenmeyer flask rinsing graduated cylinder with three 5 ml portions of CO_2-free H_2O, add 1 drop phenol red indicator soln, and titrate with 0.01 *N* $Ba(OH)_2$ in CO_2 free atmosphere. As end point is approached, stopper flask, and shake vigorously to completely extract the acid from solvent phase. Correct titration for blank by applying titration of corresponding volume of percolate obtained from a separate column run including all the reagents but omitting the sample. One ml 0.01 *N* $Ba(OH)_2$ equals 0.9 mg lactic acid.

(*C*) *Identification of lactic acid:*

Separate aqueous layer in titration flask, add 0.01 *N* $ZnSO_4$ equivalent to $Ba(OH)_2$ used in titration, heat to boiling and boil 5 min., stir in small amount of Norite, filter, and evaporate to dryness on steam bath. Add 10 ml acetone, heat to boiling, and decant, discarding the acetone. Repeat heating and decanting once more with acetone, then once with alcohol. Dissolve residue in small volume of H_2O, filter if soln is not clear, and evaporate on steam bath to concentration of 5 mg lactic acid per ml. Place few drops of soln on microscope slide, allow H_2O to evaporate at room temp., and observe crystals. For amounts of lactic acid less than 5 mg, allow soln in beaker to evaporate at room temp., then place beaker under microscope and examine crystals. Compare crystals with those obtained from a known soln of pure zinc lactate.

LACTIC ACID IN DRIED MILKS AND DRIED EGGS

PREPARATION OF SAMPLE

(*a*) *Dried whole and skimmed milks.*—Weigh 5 g into 100 ml beaker and with heavy stirring rod make into smooth paste with H_2O. Transfer contents of beaker to 100 ml volumetric flask with ca 50 ml H_2O, add 6 ml *N* H_2SO_4, mix, avoiding vigorous agitation; add 5 ml 20% phosphotungstic acid soln, make to mark with H_2O; shake, and filter thru folded filter paper. Transfer 50 ml of filtrate to 100 ml beaker and evaporate to 10 ml.

(*b*) *Dried eggs.*—Weigh 5 g into 100 ml beaker and with a heavy stirring rod make into smooth paste with H_2O. Transfer to 200 ml Erlenmeyer flask, make weight to ca 70 g with H_2O, add 10 ml *N* H_2SO_4, mix well, add 12 ml 20% phosphotungstic acid soln, make to 125 g with H_2O, shake 1 min., and filter thru folded filter paper (50 ml of filtrate will be found to weigh 50 g). Transfer 50 ml of filtrate to 100 ml beaker and evaporate to 10 ml.

EXTRACTION OF LACTIC ACID AND PREPARATION OF BARIUM LACTATE

Place 20 g $(NH_4)_2SO_4$ into dry extractor. Transfer the evaporated material into the inner tube of extractor thru a small funnel with sufficient H_2O to make total volume 40 ml, and add 0.5 ml H_2SO_4 (1+1). Mix by raising and lowering inner tube. Rinse beaker with 50 ml ether into inner tube of extractor. Connect efficient condenser and proceed with extraction as directed under sec. **22.12**, placing 150 ml ether in the extraction flask containing a few grains of carborundum or glass beads and extracting the material for 3–4 hours, or for whatever period is found necessary to get complete extraction.

To flask containing ether extract add 5 ml H_2O and expel ether on steam bath. Using a 5 ml Mohr pipet, neutralize contents of flask with saturated $Ba(OH)_2$ soln (phenolphthalein indicator) adding 0.5 ml excess, and adjust volume to 20 ml with H_2O. Add 90 ml alcohol, heat almost to boiling on steam bath, cool, add ca 0.5 g filter aid, and filter with suction thru funnel with fritted glass disc (porosity C), or Caldwell crucible with asbestos mat, catching filtrate in 250 ml beaker. Rinse flask with three 5 ml portions of diluted alcohol (9+2) and transfer rinsings to funnel or crucible, sucking dry after each addition. Evaporate filtrate to ca 5 ml

on steam bath, then transfer the small volume to a 50 ml beaker, rinsing the 250 ml beaker with H_2O, and continue evaporation to dryness.

ISOLATION OF LACTIC ACID

To dry residue of barium lactate in 50 ml beaker, add 3 drops H_2SO_4 (1+1), rub with stirring rod until the barium salt is thoroly wet, stir in 200 mg anhydrous Na_2SO_4, and proceed as directed under Standardization of Column (*B*) Separation technic, using a new partition column for each determination and adding 1 ml acetic acid soln in $CHCl_3$ to the column prior to addition of sample.

NOTES

A "slit-light" lamp for chromatographic work for translucent columns can be made from the RV "Black Light," 110–120 Volt 6 Watt 60 Cycle AC Lamp, manufactured by Vogel Luminescence Corporation, San Francisco, California, by removing the BL lamp and the black glass filter tube and replacing them with a daylight lamp of same wattage and a metal tube (brass of the same outside dimensions as the black glass filter tube) in which has been cut a slit 3/32 inch by 4 to 6 inches long.

The barium lactate (apparently none available commercially) was prepared by the following procedure:

Dilute 50 ml lactic acid with 200 ml H_2O, heat to boiling and add excess of $BaCO_3$ in small quantities to the boiling solution. Stir in ca 0.5 g Norite and 1 g filter aid and filter thru a folded filter paper, refiltering if necessary. Evaporate filtrate on steam bath to a heavy syrupy condition and complete the drying with occasional stirring at 100–105°. Pulverize the cake, add ca 150 ml (9+2) alcohol, heat to boiling on steam bath, cover with watch glass, stir and add small quantities of (9+2) alcohol to the boiling liquid until the barium lactate just dissolves. Cool with occasional stirring to room temperature, or overnight. Stir, filter thru a fritted glass funnel or crucible (porosity C), and continue suction until the barium lactate is apparently dry. Redissolve in (9+2) alcohol and repeat the crystallization. After filtration, wash the crystals in the funnel or crucible three times with about 20 ml alcohol continuing the suction after each rinsing until apparently dry. Spread the barium lactate on watchglass and dry at 100–105° overnight.

No lot of lactic acid thus far tested has been found to be chromatographically pure. It can be tested as follows:

In a small beaker containing 2 ml solvent (20% tertiary butanol in chloroform), add 1 drop lactic acid and 3 drops (1+1) sulfuric acid, mix, transfer immediately to column and sink solution into the silicic acid gel. Fill tube with solvent and develop chromatogram as usual.

It is necessary to precede the lactic acid with another acid to free the column from any excess ammonia. When less than ca 3 mg lactic acid is present, the band may not be distinct enough to be visible even if the column is viewed against a strong "slit-light." In such case, remove and discard the percolate equal to the threshold volume for lactic acid established for the silicic acid being used.

To determine the time necessary to extract all the lactic acid, transfer 2 ml of the Stock Solution, equivalent to 10 mg lactic acid, to extractor containing 20 g $(NH_4)_2SO_4$, adjust total volume in extractor to 40 ml, add 0.5 ml (1+1) H_2SO_4 and proceed with the extraction as described. To determine quantity of lactic acid extracted, add 10 ml water to extraction flask, evaporate ether on steam bath and titrate with 0.01 *N* $Ba(OH)_2$. Three hours is usually sufficient to give recovery of 95% or better. In case it is not enough time, start with another 10 mg lactic acid and extract for a period of time that will insure recovery of 95% or better.

The preparation of sample of dried milks, ether extraction, and preparation of barium salts are described in *Methods of Analysis* (1945), secs. **22.8, 22.12,** and **22.13,** respectively. The preparation of sample of dried eggs is described in *This Journal,* **27,** 210(1944). They are included here as a matter of convenience.

In the cover letter accompanying the samples to the collaborators it

was suggested that if difficulties are encountered on samples 3 and 4, make another ether extraction of the samples and remove the ether by transferring the ether extract to a flask which will stand vacuum, connect the flask to a water pump, evaporate ether at room temperature and continue vacuum one hour after removal of ether. Add 5 ml water and pro-

TABLE 1.—*Collaborative results*

COLLABORATOR	BARIUM LACTATE (10 MG LACTIC ACID USED) SAMPLE NO. 1	NONFAT DRY MILK SOLIDS		DRIED WHOLE EGGS SAMPLE NO. 4
		SAMPLE NO. 2	SAMPLE NO. 3	
	mg lactic acid	*mg lactic acid per 100 g sample*		
Hyman D. Silverberg*	6.49 6.45	0.24	2.60	0.50
H. C. Van Dame	— —	126 120	1050 1029	13 17
Fred Hillig	9.0 8.7	54.8 54.0	909 1060	150 162
A. Lada	8.8 9.0 9.1	55.2 51.5	912 924	124.8 121.6
Sylvia Shendleman	9.1	19.4 16.5	1181 1184	94.6 89.9 80.1[a]
Leslie W. Ferris	8.8 8.7	21.6 24.8	1104 881	12 73[b]
Abram Kleinman	9.3[c] 9.2[c]	54.0[d] 49.2[d]	1066 1028	116
Sidney Williams	7.1[e] 8.1[e] 8.1(8.6)[f]	44[f]	470[f]	115[f]
H. W. Conroy	9.5	31 29	1177 1158	67 76
Maximum	9.5	126.0	1184	162
Minimum	7.1	16.5	470	12
Average	8.8	50.1	1009	87

* Results omitted from maximum, minimum, average and discussion.
[a] Was treated by the alternate method of evaporating the ether extract at room temperature under water vacuum. No improvement was noted in this method.
[b] Vacuum treatment was used to remove the ether.
[c] Recovery based on titration of 100 ml of percolate.
[d] Results based on titration of 75 ml of percolate.
[e] Result based on titration of 50 ml of percolate.
[f] Results based on titration of 75 ml of percolate.

ceed as directed in the mimeographed method. This step may be necessary if the samples contain more than a trace of formic acid.

COMMENTS OF COLLABORATORS

H. C. Van Dame.—On samples 2 and 3, Dried Skim Milks, no difficulty was encountered. The lactic acid band was plain and came off at the right place. On sample 4, Dried Whole Eggs, one determination gave no band. The other gave a band which did not come off until after lactic acid should have been removed. The titrations were so small that only a very small amount of lactic acid, if any, seemed to be present.

Fred Hillig. Sample 1, Barium Lactate.—In the modified method sufficient sodium sulfate was added to thoroly dry the material in the beaker (approximately 1 g). After decantation onto the column the residue was transferred to the top of the column and the beaker was again washed with the solvent. This procedure gave practically quantitative recoveries, as the amount recovered was increased from 90 and 87% to 98 and 97.6%. In my work on succinic acid I also found that better recoveries were obtained if the entire contents of the beaker was transferred to the top of the column.

Sample 2 and 3, Dried Skim Milk.—The method submitted was followed exactly. The checks on sample 3 are not entirely satisfactory. This may be due to difficulty in completely transferring the acid to the column by decantation. When the extracted material is neutralized with saturated barium hydroxide the instructions call for the addition of 0.5 ml in excess. . . . When the alcoholic filtrate is evaporated to dryness in the 50 ml beaker the barium salts cling to the sides of the beaker making it very difficult to wet them with 3 drops of (1+1) sulfuric acid. Again 200 mg of sodium sulfate produces a gummy mass in the beaker making it difficult, if not almost impossible, for the solvent to thoroly penetrate this mass in order to extract out the acid for subsequent decantation onto the column.

Sample 4, Dried Whole Eggs.—Two bands were obtained. The threshold volumes were very close together and it is doubted if a good separation was made. The titrations of the two bands have been combined and computed as lactic acid.

A. Lada. Sample 1, Barium Lactate.—Closer to 1 g of Na_2SO_4 should be added to the acidified Ba salt, and this in turn added to the column with the last 1 ml washing of the beaker. Two determinations run in this manner with the same stock solution gave recoveries of 96 and 98%.

Sample 2, Dried Skim Milk.—Three bands on column. One very faint, came down the column very slowly and did not interfere with the determination.

Sample 4, Dried Whole Eggs.—One faint band came down the column very slowly. No interference two additional distinct bands. However, these appear to be two different materials, judging from the separation of the bands. The addition of 0.5 ml of saturated $Ba(OH)_2$ (after the evaporation of the ether from the extraction), leads to large excess of Ba salts in the final evaporation of the Ba lactate. This is difficult to wet with 3 drops of (1+1) H_2SO_4. One drop of $Ba(OH)_2$ in excess after neutralization would appear to be sufficient. As mentioned under the standardization of the column, about 1 g Na_2SO_4 should be added to the acidified Ba salt and then added to the column with the final washing.

Sylvia Shendleman.—9 to 10 pounds of pressure could not be applied to the partition column. 4 to 5 pounds were ample to force the excess liquid out of the column. In samples 2 and 3, Dried Skim Milk, and 4, Dried Whole Eggs, one or two additional drops of saturated barium hydroxide were added to the solution containing the barium lactate before evaporation to dryness. The pink color disappeared on heating, and to obtain a red residue the excess alkali was added.

The 0.01 *N* solution of barium hydroxide was stored in a paraffin lined bottle protected with a soda-lime absorbent tube and contained a siphon tube to reduce the possibility of contamination with CO_2. A soda-lime tube was also placed in the burette immediately after filling. Unless these precautions are taken, a film forms in the burette making it unusable.

The acetic acid and lactic acid bands were diffused, and the boundaries were not sharp.

The beaker containing the residue of barium lactate had a noticeable white deposit near the top. It was difficult to wet this down with the 3 drops of H_2SO_4 (1+1).

L. W. Ferris.—Sample 2, Dried Skim Milk, and Sample 4, Dried Whole Eggs, gave a band following the acetic acid band which was visible at the top portion of the column but as it moved down to approximately the center of the column it was no longer visible.

During removal of the ether (from the ether extract in lieu of adding water and evaporating ether on steam bath) by vacuum from the water pump moisture collected on the outside of the flask cooling it so that half a day was required to remove the ether. Would suggest that if this step is necessary that it be made part of the procedure and read "immerse the flask in a waterbath held at —°C."

Abram Kleinman. Sample 1, Barium Lactate.—Standardization of the chromatographic column was performed according to paragraph **(b)** of the method. Two runs were made, in which 50 ml of percolate were collected. Results 8.9 and 8.5 mg lactic acid recovered of the 10 mg added. The rather low recoveries caused me to wonder whether an appreciable amount of the lactic acid may not still remain in the column after collection of the 50 ml of percolate. Therefore, two more runs were made in which additional amounts of percolate were collected after the first 50 ml. Results, cumulatively, were for 50 ml percolate, 8.8 and 8.8 mg; for 75 ml, 9.2 and 9.1; and for 100 ml, 9.3 and 9.2 mg; and an additional 25 ml of percolate increased the lactic acid recovery by about 4%. All these figures are corrected for a column blank equivalent to 0.13 mg lactic acid. This column blank was obtained on 50 ml of percolate. Additional 25 ml percolate on the blank was negligible. All subsequent figures are similarly corrected for the column blank. Threshold volume for the standard lactic acid was 28 to 29 ml.

Sample 2, Dried Skim Milk.—Determinations were made on 50 ml and 75 ml of percolate in duplicate, finding 44.8 and 40.0 mg lactic acid/100 g dried milk for 50 ml of percolate and 54.0 and 49.2 mg, respectively, for 75 ml. Since the band rapidly became invisible, the percolate for lactic acid was collected starting at a threshold volume of 28 ml.

Sample 3, Dried Skim Milk.—Results are reported for 50 ml of percolate only. An additional 50 ml of percolate increased lactic acid recovery by only 2.3%. The lactic acid band appeared as one solid band on the column. Vacuum treatment for removal of formic acid was, therefore, not indicated.

Sample 4, Dried Whole Eggs.—Two runs without vacuum treatment after ether extraction showed 2 bands on the column close together, in the vicinity where the lactic acid would be expected. Another run was made using the vacuum treatment to remove formic acid. This treatment largely eliminated one of the bands (a small, slow moving band was observed above the lactic acid, which did not interfere with the removal of the latter). However, to avoid any possible confusion due to this slow moving band, only 50 ml of percolate was collected. Threshold volume observed, 25 ml.

Microscopic test for zinc lactate crystals yielded star-shaped clusters of needle-like crystals radiating out from the center of the star, in the dried milk and dried egg samples. A sample of barium lactate carried through the column procedure

produced similar crystals in star-like clusters and, in addition, yielded circular rosettes of needle-like crystals. The crystals obtained by adding an equivalent amount of zinc sulfate to the standard barium lactate soln were formed in circular rosettes of needle-like crystals.

Sidney Williams.—In each of the samples it appeared that the lactic acid was not completely removed from the chromatographic column by the first 50 ml of percolate following the threshold volume, therefore, after collecting and titrating 50 ml percolate an additional 25 ml was collected and titrated.

Using the crystals of zinc lactate obtained from Sample 1, Barium Lactate, as authentic, zinc lactate crystals were prepared and identified from the percolate of samples 2, 3 and 4.

Recovery of lactic acid through the column seems to be poor since it ran from 70.8% to a top of 86.1% in 75 ml of percolate. Recoveries were calculated assuming that sample 1 is pure barium lactate and that the prepared stock soln actually contained 5 mg lactic acid per ml.

Is the distillation of the ether and alcohol from NaOH actually necessary? It is inconvenient and time consuming and previous methods have not called for it.

H. W. Conroy. Sample 1, Barium Lactate.—10 mg lactic acid used and 9.5 mg recovered on chromatographing. Recovery 95%.

Sample 3, Dried Skim Milk.—Removal of formic acid by evaporation of ether at room temperature, in vacuum flask, did not affect result appreciably.

Sample 4, Dried Whole Eggs.—After removal of formic acid by above method, a lower value for lactic acid was obtained. Only one determination made by each procedure. No interference was encountered in either of samples 3 or 4 as far as could be seen on the column.

Identity test for lactic acid showed it present in all four samples.

DISCUSSION

In general the results are only fair.

The recovery of lactic acid from barium lactate averages 88%, which is somewhat low. Hillig and Lada obtained practically quantitative recoveries by increasing the amount of anhydrous sodium sulfate from 200 mg to about 1 g. The purpose of the sodium sulfate is to remove H_2O from the 3 drops (1+1) sulfuric acid added to free the lactic acid from barium lactate because H_2O is a better solvent for lactic acid than the solvent used to develop the chromatogram. Yet the amount of water in the 3 drops of (1+1) sulfuric acid appears to be soluble in the 5 ml solvent used to dissolve and transfer the lactic acid to the column. It is quite possible that the gummy mass formed when only 200 mg of sodium sulfate is used retains some of the lactic acid. Some of the collaborators may have transferred the gummy mass of sodium sulfate to the column. If so, probably this may explain why some of the collaborators increased their recoveries by collecting and titrating much larger quantities of the percolate than normally required since the lactic acid retained in the gummy mass is very slowly removed by the solvent. Increasing the amounts of sodium sulfate to 1 g prevents the formation of a gummy mass. More work appears necessary to establish whether or not anhydrous sodium sulfate is required in the method.

Again, since acetic acid is passed through the column ahead of lactic acid to remove any excess ammonium hydroxide, it might be that for some unknown reason the acetic acid failed to remove it and this might be the cause of lower recovery of lactic acid. One collaborator, H. I. Macomber, who has made no report at the time this is written, wrote that "I have been having trouble with the preparation and standardization of the partition column for lactic acid. In the first place, I have found that the use of *N* NH_4OH in the preparation results in a column so alkaline that the acetic acid band disappears entirely before it reaches the bottom of the tube. After much experimentation I obtained best results by using an indicator made without any NH_4OH and the use of only one drop of 0.1 *N* NH_4OH to 4 ml of indicator. In the second place, my yields of lactic acid are rather erratic, varying from about 88 to 90%, with an average of only 88.7% for four determinations. I thought that the silicic acid might be at fault, since I had had it for several months. I have tried another lot which we had on hand with essentially the same results. I wonder if you have any suggestions as to what I should or can do to improve the yields." He was informed that other collaborators had improved the recovery of lactic acid by increasing the amount of sodium sulfate from 200 mg to about 1 g and that there was some question as to the need of adding ammonium hydroxide and then later take steps to remove it by adding a faster moving acid ahead of the one to be determined, as it does not seem logical.

The wide variation of results reported by the collaborators for lactic acid found in the two samples of dried skim milk and one sample of dried whole eggs indicates that the ether extraction of the samples was probably incomplete. The *Book of Methods,* **22.12,** directs "continue extraction until equivalent of 7500 ml of ether has passed through solution being extracted." As the work was done during the warmest part of summer, it may be that the high temperature of the water passing through the condenser slowed the extraction materially and that the statement in the method of "extracting the material for 3–4 hours, or for whatever period is found necessary to get complete extraction" may have been misleading, causing a shorter time of extraction than necessary.

ACKNOWLEDGMENTS

The Associate Referee expresses his gratitude to the collaborators of the various Districts, Food and Drug Administration, for their generous efforts.

The Associate Referee recommends* that the study be continued.

* For report of Subcommittee C and action of the Association, see *This Journal,* 34, 49 (1951).

REPORT ON FISH AND OTHER MARINE PRODUCTS

By ANDREW M. ALLISON (Food and Drug Administration, Federal Security Agency, Kansas City 6, Missouri), *Referee*

The Associate Referee on Total Solids in Fish has done some preliminary work showing promise, but it has not progressed sufficiently to warrant a report.

In decomposition studies, work on the application of several chemical indices is being continued with codfish samples.

RECOMMENDATION*

It is recommended—

(1) That work be continued on methods for determination of total solids in fish.

REPORT ON SPICES AND OTHER CONDIMENTS

By E. C. DEAL (U. S. Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Referee*

Last year Subcommittee C approved recommendations for the study of nine topics in this field. Four Associate Referees have been appointed and six of the topics fall within their provinces. The Referee received reports from three Associate Referees.

VINEGAR

The Associate Referee submitted a report covering investigational studies of the application of the Permanganate Oxidation Number to samples of commercial and authentic vinegars. His studies indicate that the usefulness of this determination in determining whether or not glacial acetic acid has been added to distilled or cider vinegar is open to question. This seems to be particularly true when the vinegar has been produced by the modern Frings process. The method as published in *This Journal*, **32** (1949) was recommended for adoption as official. The Associate Referee reports that he has started certain collaborative studies on the reproducibility of the method. The Referee recommends* that these be continued.

Owing to the large number of topics included in the study of vinegar, the Associate Referee recommends that other Associate Referees be appointed and assigned certain phases of the work. The Referee concurs.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 49 (1951).

PREPARATION OF SAMPLE, AND FAT IN MAYONNAISE AND SALAD DRESSINGS

The Associate Referee submitted a report on the method for preliminary treatment of fat for total nitrogen in mayonnaise and salad dressings. Her studies indicate that no material advantage is gained from a preliminary treatment of the samples with chloroform before extraction of the fat with petroleum ether. She found that some samples require more than 35 ml. of sulfuric acid to effect digestion and she recommends that this figure in the method be changed to read "50 ml.," and that a cautionary note be added that in rare instances more than 50 ml. may be necessary. The Referee concurs in these recommendations.*

SEEDS AND STEMS IN GROUND CHILI

The Associate Referee reported that a method has been developed based upon the hot water-insoluble material in ground chilies and that the method shows promise of being useful in detecting added ground seed and stem material. He recommends* that the work be continued. The Referee concurs and recommends that the program include collaborative study.

No reports were received on volatile oil in spices and on sugar, ash, and pungent principles in mustards. The Referee recommends* that these topics be re-assigned for study.

The Referee further recommends that additional Associate Referees be appointed and that study of the remaining topics recommended last year be continued.

REPORT ON VINEGAR

By G. A. MICHAEL (Dept. of Public Health, State House, Boston 33, Mass.), *Associate Referee*

The recommendations adopted at the last meeting on vinegar follow:

1. That studies of methods for the detection of caramel in vinegar be continued.
2. That studies on the determination of tartrates in vinegar be continued.
3. That the method for "Permanganate Oxidation Number," as described by the Referee, be adopted as official.
4. That methods for the determination of free mineral acids in vinegar be further studied.
5. That methods for the determination of sorbitol, and the value of these methods for the detection of cider vinegar in wine vinegar, be studied.

No work has been done in regard to the above recommendations, except in the case of recommendation number three.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 51 (1951).

The Associate Referee is in accord with the recommendation of the Committee on Vinegar (No. 3) to adopt the permanganate oxidation number as official, in its capacity as a reproducible test for the determination of steam volatile reducible substances. Its application as a means of determining whether or not glacial acetic acid has been used as an adulterant for distilled and cider vinegars is open to considerable discussion.

As a result of the work of O'Neill and Henry, and also of our work with the permanganate oxidation number, we have been viewing with suspicion all samples of cider and white distilled vinegar whose permanganate oxidation numbers fall below 3.00.

TABLE 1.—*Frings process vinegar*

	SAMPLE NO.	ACETIC ACID	TOTAL SOLIDS	PERMANGANATE NO.	THEORETICAL YIELD	YIELD	EFFICIENCY
		per cent	*per cent*				*per cent*
2/25/48 Charge	1	0.68	1.84	—	7.77	—	—
2/29/48 Out	1	7.17	1.60	2.26	—	7.17	92.3
2/29/48 Charge	2	0.63	1.80	—	7.29	—	—
3/ 4/48 Out	2	6.63	1.60	2.84	—	6.63	90.95
3/ 4/48 Charge	3	0.64	1.79	—	7.18	—	—
3/ 7/48 Out	3	6.39	1.64	2.55	—	6.39	89.0
3/ 7/48 Charge	4	0.63	1.80	—	7.18	—	—
3/ 9/48 Out	4	6.28	1.64	4.45	—	6.28	87.4
3/ 9/48 Stock	5	0.66	1.76	—	7.27	—	—
3/11/48 Out	5	6.28	1.63	4.22	—	6.28	86.4

The initial application of the permanganate oxidation number test to vinegars acquired from the open market in Massachusetts showed that of a group of 54 samples, 17 samples or 31.5%, had permanganate numbers lower than 3.00. After this investigation, it became known to the trade that a method was being used by us for the detection of commercial acetic acid in vinegar.

A very good case in favor of the creditability of the results was seen when one packer admitted that he used acetic acid, and also found it in the analysis of the next lot of vinegars purchased on the open market six months later. The second lot of 36 samples contained only 4 samples, or 11.12 per cent whose permanganate oxidation numbers were lower than 3.00.

Recently, we purchased six samples of vinegar on the open market, four of which were from concerns from whom we had obtained samples which had low permanganate numbers. The permanganate numbers varied from 5.37 to 9.21 (see Table 2).

Five lots of vinegar were obtained from a Massachusetts manufacturer who uses the Frings process. In each case we acquired the cider "charge"

TABLE 2.—*Vinegar bought on open market*

SAMPLE NO.	ACETIC ACID	PERMANGANATE NO.
	per cent	
1	4.09	5.37
2	4.16	6.24
3	4.03	9.00
4	3.88	6.34
5	3.88	8.80
6	4.04	9.21

and the resultant vinegar product. The resulting analyses obtained certainly open a new field for thought (see Table 1). The average efficiency of the process according to these five lots is 89.37 per cent. The per cent of acid varies from 6.28 to 7.17. The permanganate number varies from 2.26 to 4.45. These figures make us hesitate in prosecuting on the strength of a minimum permanganate number of 3.00.

On the other hand, we have a lot of six samples of vinegar submitted to us by Mr. Crowell (see Table 3), which range from 4.11 to 5.03 per cent acetic acid. The permanganate numbers vary from 9.31 to 18.55. This certainly allows for considerable adulteration if solids are added in proportion to the adulteration.

A sample of white distilled vinegar was drawn from a Frings generator at the end of conversion (see Table 4). At this stage of the process, the distilled vinegar is straw-colored. It is made white by decolorizing with charcoal. At one time, I suspected that some of the low permanganate numbers of several samples of white distilled vinegar may have been due to this decolorizing process, but as the figures in Table 4 show, the decolorization does not seem to effect the permanganate number. Sample No. 2 is a final product from the same process and gives a 4.42 permanganate number.

TABLE 3.—*Samples received from New Hampshire Department of Health*

SAMPLE	ACETIC ACID	TOTAL SOLIDS	ALCOHOL	PERMANGANATE NO.
	per cent	*per cent*	*per cent/wt.*	
No. 1—hard cider	—	1.65	4.62	—
No. 2—hard cider	—	1.64	4.80	—
No. 3—hard cider	—	1.54	3.97	—
No. 4—hard cider	—	1.92	4.54	—
No. 5—vinegar	4.20	1.73	0.14	11.4
No. 6—vinegar	4.23	1.74	0.13	11.7
No. 7—vinegar	4.11	1.66	0.13	9.31
No. 8—vinegar	4.14	1.66	0.12	9.62
No. 9—vinegar	4.18	1.50	0.74	18.55
No. 10—vinegar	5.03	1.54	0.25	12.62

In my opinion, further collaborative work to determine whether or not this test will properly respond to measured adulterations is in order. Samples have been sent out to various collaborators, but results will probably not be forthcoming before the deadline set for the filing of this report.

In view of the above results, it is the recommendation of the Associate Referee that in the analysis of the permanganate number, the above facts be taken into consideration.

In regard to the presence of caramel in cider vinegar, it has long been the opinion of the Associate Referee that caramel may be produced in the cider vinegar during processing in the closed or Frings generator, if

TABLE 4.—*Distilled vinegar*

	PERMANGANATE NO.	ACETIC ACID
		per cent
Sample No. 1, Tank colored	1.69	12.38
Same decolorized	1.69	12.00
Sample No. 2, Decolorized	4.42	10.6

the cooling medium is not closely supervised; the charring of the small quantities of sugar present is due to the high temperatures caused by the uncontrolled fermentation process.

The Associate Referee has been primarily interested in determining identifiable characteristics of pure cider vinegar. This project, with its many factors, has been extremely complicated in its solution. Two of the factors which have received special attention by the Food and Drug Division of the Massachusetts Department of Public Health have been the total solids content and the permanganate number.

At one time we felt that a cider vinegar should have a total solids content in excess of 1.80%, more than 4% acetic acid, and a permanganate number higher than 3.0%, and an absence of caramel added as artificial coloring matter. Today, the closed process or Frings generator cider vinegar may run as low as 1.20% total solids, an acid figure of 7.17%, and a permanganate number as high as 28.0%; and caramel may be produced by overheating in the generator. These, of course, are maximum figures. The dilution of 7% vinegar to 4.00% legal strength in Massachusetts law may drop the solids content well below 1%. A permanganate number of 28.0 would allow for ten volumes of adulteration according to the minimum permanganate numbers set forth in the work of O'Neill and Henry.[1]

It is the hope of the Associate Referee to make certain determinations to show the characteristics or constants that may be expected in an adul-

[1] O'Neill, R. E., and Henry, A. M. *This Journal*, **27**, 263 (1944).

terated cider vinegar. Coöperating to this end is the pledge of the New England Food and Drug Officials.

The first problem is to obtain known purity samples. In order that these samples may be properly evaluated, they must be in series form. A series would contain the sweet cider, the hard cider, the unclarified vinegar, and the clarified vinegar which is used in making a certain lot of vinegar, as well as the information as to what varieties of apples were used in the process. The Associate Referee has had the offer of coöperation from three large vinegar manufacturers in New England. They have offered to supply some of the necessary samples.

Another factor that seems to effect the total solids content of cider vinegar is the weather condition which prevails during the growth of the apples. The drought of the summer of 1949 effected the apple crop of Massachusetts in such a manner as to produce a very low solids cider vinegar. The processes used today to produce the cider vinegar further complicate the problem. There are three methods in use for the production of cider vinegar in Massachusetts—the old very slow vat method; the old type open-generator process; and the newer closed type, or Frings generator,[2] the three processes each producing legal vinegar with different characteristics. This multiplies the natural variance of the raw materials by three. In the case of the vat process, there are high solids with low acid; the open-generator method produces slightly higher acid and slightly lower solids; the closed-generator method produces the lowest solids and the highest acid content.

REPORT ON SEEDS AND STEMS IN GROUND CHILI

By A. N. PRATER (Gentry, Inc., Los Angeles 54, Calif.), *Associate Referee*

Standard commercial practice in the preparation of chili pepper and chili powder is to grind whole pods of various chili varieties containing their normal component of seeds and stems. These products may be upgraded by either the partial or complete removal of the seeds and/or stems and, conversely, lower quality products can be made by the addition of seeds and stems in amounts above those normally present. Addition of seeds and stems in amounts above those normally present may constitute adulteration under State or Federal Laws.

A method is needed for the quick and easy determination of the seed and stem content of ground chili products in order to determine whether seed and stem material is present in normal amounts or whether it has been added or removed.

A method has been developed, based upon the hot water-insoluble material in ground chilies, and which consists essentially of determining

[2] Michael, George A., Vinegar Analysis, Association of Food and Drug Officials of the United States, Quarterly Bulletin, July, 1949.

the per cent insoluble in hot water and relating this percentage to authentic samples of material. Significant differences occur between hot water-insoluble content of seeded and stemmed material, on the one hand, and whole pod material, on the other. Additional data are being obtained and the method is being correlated with a microscopic identification technique.

It is hoped to have a contributed paper ready within the next few months. It is recommended* that work be continued on this method.

REPORT ON FAT AND TOTAL NITROGEN IN MAYONNAISE AND SALAD DRESSING

By JUANITA E. BREIT (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The efficiency of the method for preliminary removal of fat in the official method for total nitrogen in mayonnaise, 33.52, was checked and

TABLE 1.—*Nitrogen in mayonnaise and salad dressings*

SAMPLE	PRODUCT	FAT CONTENT	NITROGEN CONTENT	
			A.O.A.C. METHOD	A.O.A.C. METHOD MODIFIED
		per cent	*per cent*	*per cent*
1	Mayonnaise	84.4	0.221	0.215
2	Mayonnaise	80.1	0.175	0.174
3	Salad dressing	19.9	0.094	0.097
4	Salad dressing	23.6	0.092	0.084
5	Dietary dressing	10.4	0.308	0.306
6	Dietary dressing	7.6	0.115	0.115
7	Dietary dressing	12.5	0.178	0.175
8	Dietary dressing	16.1	0.286	0.287
9	Salad dressing	18.9	0.085	0.084
10	Salad dressing	36.1	0.207	0.207
11	Mayonnaise	75.9	0.021	0.020
12	Salad dressing	26.8	0.220	0.221
13	Salad dressing	28.2	0.083	0.082

studied further in accordance with the recommendation of subcommittee C.[1]

Several years ago difficulty was reported when using the A.O.A.C. method for removal of fat from mayonnaise prior to acid digestion for nitrogen determination. It was stated that, for some samples analyzed, more than 50 ml of H_2SO_4 and two days of heating were required for complete digestion. Assuming the difficulty to be due to incomplete extraction of fat before digestion, the following procedure was suggested:

* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 51 (1951).
[1] *This Journal*, 33, 57 (1950)

1. Add ca 100 ml of chloroform to the sample in the Kjeldahl flask.
2. Shake to get a homogeneous mixture (swirling is preferable because in shaking part of the sample will get up into the neck of the flask).
3. Place on the steam bath and evaporate the chloroform.

When this method was suggested it was felt that as the chloroform evaporated, the egg proteins would precipitate, facilitating fat removal.

Thirteen samples of mayonnaise, salad dressing, and dietary dressings were analyzed using both the A.O.A.C. method and the same method modified with chloroform. The dietary dressings, some containing gums, were purchased in a health food store. Results of the analysis are shown in Table 1.

DISCUSSION OF RESULTS

Only in samples of high fat content was it possible to obtain a homogeneous mixture by swirling the Kjeldahl flask after the addition of chloroform; in those of low fat content the product clumped or floated on the surface of the solvent.

When petroleum ether was added to the chloroform-treated samples, various reactions occurred. The high fat content samples dispersed and were easily extracted, except for one mayonnaise which disintegrated into a powdery mass, most of which remained suspended in the ether. In decanting this sample, extreme care had to be used to prevent the loss of part of the non-fat protein. The other samples formed into a gummy, hard, or hard and almost brittle clump, making intimate contact of all parts of the sample with the ether impossible. Of the non-chloroform-treated samples, those of high fat content dispersed readily; of the low fat samples, some clumped but were more soft, not quite as gummy, and not hard or brittle, permitting somewhat better contact between sample and solvent.

For acid digestion, sample 9, method 1 required about 60 ml of sulfuric acid and method 2, required 40–45 ml; for samples 3, 4, 12, method 1 required 40 ml; 35 ml of acid were sufficient for all the other nitrogen determinations. The length of time needed to effect complete digestion of all samples was approximately the same for both methods, 3 hours and 15 minutes being the longest time, including 2 hours of heating after the solution was colorless.

CONCLUSIONS

(1) For nitrogen determination of salad dressing or mayonnaise, a preliminary treatment with chloroform is unnecessary.

(2) Some samples require more than 35 ml of H_2SO_4 to effect complete digestion, 50 ml being sufficient in almost all cases.

RECOMMENDATIONS*

It is recommended—

(1) That the official method for total nitrogen in mayonnaise and salad dressing, **28.44,** be amended to substitute 50 ml of H_2SO_4, instead of 35 ml.

(2) That a cautionary notation be added that in rare instances an excess of 50 ml of H_2SO_4 may be necessary.

REPORT ON CEREAL FOODS

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

RECOMMENDATIONS†

It is recommended—

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

(2) That the study of modification of method, sec. **13.30, 13.31,** and **13.32,** for the determination of reducing and non-reducing sugars in bakery products, as recommended in the Associate Referee report for last year, be continued.

(3) That the method for benzoic acid in flour in the Associate Referee report last year be made official.

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

(5) That the work for the determination of proteolytic activity of flour be continued.

(6) That the study on methods on soybean flour, for moisture, ash, nitrogen, crude fiber, and oil or petroleum benzine extract, be continued.

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

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

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

(10) That the procedure presented by Associate Referee on the determination of moisture (loss in weight by drying), in flour products containing sodium bicarbonate as one of its constituents be adopted as first action.

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

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* For report of Subcommittee C and action of the Association, see *This Journal,* 34, 51 (1951).
† For report of Subcommittee D and action of the Association, see *This Journal,* 34, 53 (1951).

(12) That the procedure of the Associate Referee on the determination of acetic and propionic acid in bread be adopted, first action, and the study continued.

(13) That the sec. **13.43 (a)** and **(b)** and **13.44,** p. 204 (first action) be dropped and that Method I of the report on nitrites in flour be adopted as first action.

(14) That the sec. **13.118** and **13.119** (first action) be dropped and the procedures of the report on carotene in noodles be adopted as first action.

REPORT ON STARCH IN CEREALS

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

In principle, the Rask[1] method for starch in cereals is the simplest, most specific, and most rapid of any available method. However, the practical application of the Rask method and various modifications for cereals have not had general acceptance. During the past year the Referee and Edward F. Steagall, Washington, D. C., of the Food and Drug Administration, have studied the application and comparison of five (5) methods and various modifications. Based on a background of experience with the original Rask method and various modifications, the conclusions were drawn that the application of the Rask-McVey[2] method in practice should at least closely conform to theory. Accordingly, the following Rask-McVey procedure was adapted to cereals and submitted to eight collaborators.

DETERMINATION OF STARCH

Place exactly 1 g sample into 125 ml Erlenmeyer flask, add 2 g filter-cel or dicalite (rough balance weight), add 50 ml 4% alcoholic KOH (4 g KOH per 100 ml alcohol), heat on steam bath for 30 min. with flask fitted to reflux condenser, shake occasionally. Fit filter paper (S&S 589 white ribbon) conveniently cut out with cork borer, into a Gooch crucible, transfer sample with aid of stream of alcohol, and continue to wash until the total volume in the suction flasks amounts to 100 ml. Continue to dry a few min. by suction to remove most of the alcohol from the sample. Invert crucible over 150 ml beaker and transfer sample by use of stirring rod. Pour 60 ml of 5.7 *N* HCl (100 ml HCl+100 ml H_2O, mixed, cooled and adjusted by titration) into 100 ml graduate. Add a small amount of this acid (ca 8 ml) to the sample, stir to form a thick smooth paste free of lumps, continue to add small amounts of acid, and stir to a thin suspension, by using a total of ca 25 ml of the acid, and pour it into 100 ml volumetric flask (preferably a Kohlrausch flask). Rinse out the flask, Gooch crucible, and beaker with the remaining portion of the original 60 ml of acid, using small successive portions. Rinse flask and Gooch crucible only once. This mixing and transferring should take ca 5 min. Allow the stoppered flask to stand at room temp. for 15 min. with frequent shaking. Make to volume with H_2O and add 1 ml more H_2O from Mohr pipette to correct for volume of filter-cel. Mix well, filter thru Büchner funnel (50 mm diam.) fitted with filter

[1] *This Journal*, **10**, 108 (1927); *Methods of Analysis*, 6th Ed., p. 249.
[2] *This Journal*, **24**, 928 (1941).

TABLE 1.—*Results for per cent of starch by the modified Rask method*

	FLOUR	BREAD	GRAHAM CRACKERS
(1)	64.6, 66.1, 65.4, 66.2, 65.20 av. 65.5	57.5, 56.6, 56.6, 56.0, 56.3 av. 56.6	48.0, 47.6, 48.0, 47.2, 47.4 av. 47.6
(2)	61.2, 61.9, 61.2 av. 61.4	45.6, 45.6, 46.0 av. 45.7	41.9, 42.3, 41.8 av. 42.0
(3)	66.6, 67.0, 67.8 av. 67.1	56.4, 56.0 av. 56.2	45.5, 44.6 av. 45.0
(4)	68.4, 69.8, 67.3 av. 68.5	54.0, 54.0 av. 54.0	49.3, 49.1 av. 49.2
(5)	67.6, 68.7 av. 68.2	54.7, 55.4 av. 55.1	47.0, 48.4 av. 47.7
(6)	67.1, 66.5, 66.4 av. 66.7	54.8, 54.9, 55.0 av. 54.9	49.3, 48.9, 48.8 av. 49.0
(7)	68.4, 68.5, 68.2, 68.1 av. 68.3	56.2, 56.7 av. 56.4	50.2, 50.0 av. 50.1
(8)	68.5, 69.4, 68.6, 68.3, 68.9, 69.0, 68.5, 68.9, 68.2, 68.0 av. 68.6	57.6, 57.6, 57.3, 57.7, 56.4, 57.0, 57.0 av. 57.2	49.9, 50.1 av. 50.0
Max.	68.6	57.2	50.0
Min.*	61.4	45.7	42.0
Av.	67.6	55.8	48.4

* No. 2 omitted from the average.

paper (S&S 589 white ribbon) into 250 ml suction flask. Pipette 50 ml immediately into 115 ml alcohol in a 250 ml beaker, transfer carefully the filter-cel by inverting the previously dried and weighed Gooch crucible containing an asbestos pad and loosely filled ⅔ full of filter-cel. (These crucibles should be prepared well ahead of the determination and dried to constant weight, ca 2 hrs. at 130°C.). Mix with stirring rod, allow to settle a few min., transfer starch and filter-cel to the same Gooch crucible by use of suction; after nearly all the sample is transferred, turn off suction and empty flask and wash with 100 ml of alcohol guided by a previously measured 100 ml marker on the flask. Dry at 130°C. for 2 hrs. Cool ca 10 min. in desiccator, charged with fresh H_2SO_4, or freshly ignited CaO or other efficient desiccant, weigh quickly. (Starch is hygroscopic and should not be exposed longer than necessary.) The increase in weight multiplied by 200 equals per cent starch. Report at least in duplicate.

(Alcohol refers to 95% alcohol thruout procedure.)

Three samples consisting of flour, air dry bread, and graham crackers were sent each collaborator. Their results are reported in Table 1.

The results of collaborator 2 are very low, possibly due, according to the analyst, to the quality of alcohol used. Results by collaborators 1, 3, 4, 5, and 6 are somewhat lower than those reported by the Referee and Steagall, 7 and 8. The maximum deviation is 5% for the flour and bread and 10% for the Graham crackers (excluding collaborator No. 2), assuming the highest values reported are essentially the true values. These lower results undoubtedly are due to incomplete contact of the dispersing HCl or too long a period for action of the HCl before precipitation of the starch in alcohol, or both. This is a very critical and necessary part of the procedure. Possibly a note emphasizing the absolute need to keep within the time limits of the procedure would result in improvement in results.

The results on these same samples of flour and bread by 4 other methods are given for comparison.

TABLE 2.—*Comparative results for 5 different methods*

	FLOUR	BREAD
	per cent	*per cent*
Diastase Acid Method[1]	67.6	58.9
	68.2	58.9
	Av. 67.9	58.9
Polarimetric Method[2]	68.2	60.8
	68.2	60.8
Direct Acid Hydrolysis Method[3]	68.0	60.0
	68.4	61.6
	Av. 68.2	60.8
Steiner Method[4]	67.0	58.0
	67.8	58.2
	Av. 67.4	58.1
Modified Rask-McVey Method (this report)	Av. 10 results 68.6	Av. 7 results 57.2
	Av. of all 5 methods	68.1 59.2

[1] *Methods of Analysis*, 6th Ed. p. 410, Sec. **27.35.**
[2] Ibid., p. 250, Sec. **20.47.**
[3] Ibid., p. 410, Sec. **27.33.**
[4] *J. Ind. & Eng. Chem., Anal. Ed.*, **16**, 736 (1944).

The assistance of these collaborators are greatly appreciated:

Rae H. Harris, Agri. Expt. Station, Fargo, N. Dak.
Robert Frey, Red Star Yeast and Produce Co., Milwaukee, Wis.
Wm. L. Haley, Fisher Flouring Mills Co., Seattle, Wash.
Edwin L. Sexton, Best Foods, Inc., New York 17, N. Y.

R. T. Bohn, General Baking Co., New York 17, N. Y.
Niles H. Walker, Arnold Bakers, Inc., Port Chester, N. Y.
Edward F. Steagall, Food and Drug Administration, Washington, D. C.

RECOMMENDATION*

It is recommended—

(1) That the study of methods for starch in cereals be continued.

REPORT ON NITRITES IN FLOUR

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

Oxides of nitrogen is one of the permitted bleaching agents in the standard for flour. The *Methods of Analysis* of the A.O.A.C. contains on p. 204 of the 7th Edition a choice of two procedures for determination of the amount of nitrogen as nitrites in flour. These are old procedures used in the early days of the use of nitrogen peroxide bleaching. There appears to be no record of any collaborative study by the Association. Rider and Mellon[1] have reported the effect of concentration, order of addition of reagents, *p*H, temperature, light, and nitrite concentration on the colorimetric determination of nitrites by diazotization and coupling reactions with sulfanilic acid and alpha naphthylamine. Evidence reported by Rider and Mellon has been applied to the determination of nitrites in flour resulting in the Method II given in this report. Method I of this report is essentially sec. **13.44 (a)** of the 7th Ed. *Methods of Analysis*. Method II has the advantage of reactions at room temperature rather than 40° in water bath, and a more intense color at a given nitrite level. While obtaining collaborative results on the A.O.A.C. Method I it seemed wise to offer Method II for comparison. Accordingly, 3 samples of flour were sent to collaborators with the request that they analyze them by both methods.

METHOD I

Select a series of 100 ml volumetric flasks of uniform dimensions and color (125-ml Erlenmeyer flask can be used). Place 2 g of untreated (nitrite-free) flour in each flask. Add to each flask, except one for blank, varying quantities of the standard $NaNO_2$ soln (C); usually 0, 5, 10, 15, 20, 25, 30, 35 ml cover probable nitrite content of unknown sample. Add sufficient H_2O to make total volume in each flask 80 ml. Shake while adding the standard soln and H_2O to get the flour moistened and well dispersed before too dilute. Add 2 g of unknown flour to a similar flask, and add 80 ml H_2O. (Treat all flasks the same.) Digest all the flasks in water bath at 40°C. for at least 15 min, add 2 ml sulfanilic acid soln (dissolve 0.5 g sulfanilic acid in 150 ml of 20% acetic acid, warming slightly if necessary) from a Mohr pipette to each flask in succession, mix well, add 2 ml alpha naphthylamine hydrochloride soln (dissolve 0.2 g of the salt in 150 ml of 20% acetic acid, by heating, if necessary). Continue digestion at 40°C. for 20 min from time of addition to

* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 53 (1951).
[1] *Ind. Eng. Chem., Anal. Ed.*, 18, 96 (1946)

the last flask. Shake the samples occasionally during the first part of the digestion. During the latter 10 min the flour should be allowed to settle. Remove from bath without disturbing settled flour. Compare unknown with series of standards and estimate the closest match. Multiply number of ml of $NaNO_2$ soln (C) in flask by .05 to obtain p.p.m. of N. (*i.e.* unknown may be in between 30 and 35 ml, ca 32 ml, or $32 \times .05 = 1.6$ p.p.m. of N.)

METHOD II

Follow Method I to "Digest all the flasks . . ." and then change as follows: allow all flour to remain in contact with H_2O for at least 15 min at room temp, shaking occasionally; add from Mohr pipette accurately 1 ml of sulfanilic soln (dissolve 0.6 g of sulfanilic acid in ca 70 ml of hot H_2O, cool the soln, add 20 ml conc HCl; dilute to 100 ml with H_2O and mix) to each flask in succession, mix well, and allow diazotization for 6 min in diffuse light, add 1 ml of 1-aminonaphthalene hydrochloride soln (0.6 g of 1-aminonaphthalene hydrochloride and 1.0 ml of conc HCl diluted to 100 ml with H_2O) to each flask in succession, then immediately add 1 ml 2.0 M sodium acetate soln (272 g CH_3 COO Na $3H_2O$ diluted to 1.1 with H_2O) to each flask, which buffers the mixture to ca *p*H 2.4 (*p*H 2.0–2.5), allow coupling to proceed for 10 min from time of addition of sodium acetate to last flask, shake all flasks well and compare unknown with standards in 10 min after allowing flour to settle. Report in p.p.m. of N, as under Method I.

Results were obtained from 7 analysts as reported in the table.

TABLE 1.—*Nitrogen as nitrites in flour in p.p.m.*

COLLABORATOR	METHOD	SAMPLE I	SAMPLE II	SAMPLE III
1	I	None	0.6	1.2
	II	None	0.6	1.2
2	I	None	0.7	1.5
	II	None	0.5	1.3
3	I	None	0.8	1.7
	II	None	0.7	1.6
4	I	None	0.6	1.3
	II	None	0.6	1.0
5	I	None	0.6	1.5
	II	None	0.6	1.3
6	I	None	0.8	1.6
	II	None	0.8	1.6
7	I	None	0.9	1.7
	II	None	0.9	1.7

Sample I, containing no nitrogen peroxide bleach, gave a negative result by both procedures by all collaborators. The agreement on the two bleached samples is as good as could be expected on this type of colorimetric comparison and is considered satisfactory. While 5 of the 7 analysts expressed preferance for Method II, there is little difference in the results reported.

Part B, under sec. **13.44,** offers no advantages over Part A, and the two procedures do not give the same results, in the experience of the Referee.

Method I of this report, essentially *part A* of sec. **13.44,** now has satisfactory collaborative results. It is doubtful if Method II has enough merit over Method I to warrant adoption.

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Edward F. Steagall, Food and Drug Administration, Washington, D. C.

RECOMMENDATIONS*

It is recommended—

(1) That sec. **13.43 (a)** and **(b)** and **13.44,** first action, be dropped.
(2) That Method I of this report be adopted as first action.

REPORT ON CAROTENE IN NOODLES

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

The *Methods of Analysis A.O.A.C.*, on p. 218 of the 7th Edition, contain a method for the determination of carotene in flour, semolina, macaroni, and egg noodles which has not been studied collaboratively. Since this method depends on the phasic principle for separation of carotene and xanthophyll, it seemed desirable to include in any further study on this subject the more recently used chromatographic procedure. The procedure used for the isolation of the carotenoids is essentially sec. **13.119,** p. 218, of the 7th Ed., *Methods of Analysis*. After measurement of carotenoids the carotene and xanthophyll are separated. The separation is obtained in procedure A by the phasic principle similar to sec. **13.119,** and procedure B requires use of a chromatographic technic. The proposed procedures were used by 9 collaborators. Details of the methods were published in *This Journal,* **34,** 68–69 (1951).

CAROTENOID AND CAROTENE

Each analyst was asked to analyze the samples by both procedures for separation of carotene and xanthophyll on a definite date in order to minimize the effect of change in carotene content. The samples consisted of semolina, egg noodles made in laboratory containing 5.5% whole egg solids designated egg noodles #1, and a similar noodle made from some semolina and eggs plus added crystalline carotene dissolved in petroleum

* For report of Subcommittee D and action of the Association, see *This Journal,* 34, 53 (1951).

TABLE 1.—*Carotenoid pigments as carotene in p.p.m. on semolina, egg noodles, and egg noodle with added carotene*

COLLABORATOR		SEMOLINA	EGG NOODLES NO. 1	EGG NOODLES NO. 2
1	Carotenoids	5.0	6.9	9.3 – 9.9
		6.0	7.8	10.6 – 10.7
	Carotene procedure A	0.3	0.4	3.5 – 3.6
	B	0.1	0.1	3.2 – 3.2
2	Carotenoids	3.9	4.7	7.7–7.4–7.3
	Carotene procedure A	0.3	0.2	3.6 – 3.2
	B	—	—	3.2
3	Carotenoids	3.8–3.7	4.7–4.7–4.7	7.5–7.5–7.5–7.2
		3.5–3.4	4.6	
	Carotene procedure A	0.1–0.1	0.2–0.2	3.3 – 3.3
	B	0.1–0.1	0.1–0.1	3.1 – 3.1
4	Carotenoids	3.8	4.8	7.7 – 7.9
	Carotene procedure A	0.1	0.2	3.3 – 3.3
	B	0.1	0.1	2.9 – 3.0
5	Carotenoids	4.4–4.5	5.2–5.0	8.0–7.9–8.0–8.2
	Carotene procedure A	0.2	0.2	3.4 – 3.5
	B	0.1	0.2	3.0 – 3.0
6	Carotenoids	3.7–3.7	4.9–5.1	7.7 – 7.7
	Carotene procedure A	0.2–0.2	0.2–0.2	3.4 – 3.4
	B	0.1–0.1	0.1–0.1	3.1 – 2.9
7	Carotenoids	—	4.9–5.2	8.0 – 8.1
	Carotene procedure A	—	0.2–0.3	3.5 – 3.7
	B	—	0.1–0.1	3.2 – 3.2
8	Carotenoids	3.9–4.0	4.9–5.0	7.8–7.7–7.8–7.8
	Carotene procedure A	0.2	0.2	3.2 – 3.2
	B	0.03	0.1	3.2 – 3.0
Carotenoid	Max.	5.5	7.3	10.1
	Min.	3.6	4.7	7.4
	Av.*	3.9	4.9	7.7
Carotene procedure A	Max.	0.3	0.4	3.6
	Min.	0.1	0.1	3.2
	Av.	0.2	0.2	3.4
Carotene procedure B	Max.	0.1	0.2	3.2
	Min.	0.03	0.1	3.0
	Av.	0.1	0.1	3.1

* Excluding No. 1.

ether, designated as egg noodle #2. At the time of preparation, egg noodles #2 was estimated to contain 3.8 p.p.m. added carotene. It was requested that #2 be analyzed in duplicate. A sample of the absorption mixture to be used on the chromatographic separation was also submitted. The results of the collaborators are given in Table 1.

One analyst's results seem to be considerably higher for carotenoids. In general, the agreement on the rest of the results is good. The amount of carotene on the semolina and egg noodles #1 is very low by both procedures, which is characteristic for products of this type containing no added carotene. The average found for carotene in egg noodles #2 by both procedures was somewhat below the amount actually added of 3.8 p.p.m. which represents 84.2% and 79.0% recovery by procedure A and B, respectively. The recovery by the chromatographic technic is less, as indicated. No doubt the low recovery is due in large part to destruction of carotene in preparation of noodles and interval previous to analysis.

One collaborator reported results as determined by reading concentration of pigment in neutral wedge photometer as follows:

	SEMOLINA	EGG NOODLE NO. 1	EGG NOODLE NO. 2
	p.p.m.	*p.p.m.*	*p.p.m.*
carotenoids	4.2	5.2	8.6–8.6
carotene procedure A	0.2	0.3	3.1–3.2
carotene procedure B	0.1	0.1	2.5–2.4

These results are in fair agreement with those reported by use of the spectrophotometer on all other results.

A 20-g sample of semolina or macaroni does cause considerable bumping as was commented on by several analysts. The use of a 10-g sample will eliminate the bumping and also speed up filtration and at the same time give comparable results.

Several analysts reported under Method B that the volume in the suction flask after passage of the carotene thru the column exceeded the "only a few ml" referred to in the procedure. As was directed under Method A, the volume can be conveniently reduced in the suction flask under vacuum in beaker of H_2O at 45–50°C.

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F. J. McNall, Cincinnati District
L. C. Mitchell, Minneapolis District

Helen T. Hyde, San Francisco District
Edward F. Steagall, Washington, D. C.

RECOMMENDATIONS*

It is recommended—

(1) That the first action method, **13,118, 13,119,** be dropped and the method presented with this report be substituted.

(2) That the methods A and B presented in this report for carotene be adopted, first action.

REPORT ON MOISTURE (LOSS IN WEIGHT BY DRYING) IN FLOUR MIXES CONTAINING SODIUM BICARBONATE

SELF RISING FLOUR, PANCAKE FLOUR, BISCUIT FLOUR, WAFFLE FLOUR

By FRANK H. COLLINS (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

This Association for the past ten years has had under consideration the study of flour-like products containing sodium bicarbonate as one constituent. These studies have generally been captioned "Moisture in Self Rising Flour and in Pancake, Waffle and Doughnut Flours." This problem has been approached by the different Associate Referees from several angles.

The application of methods for the absolute moisture determination did not prove sufficiently satisfactory for adoption. These methods include the Karl Fisher titrimetric method and the Bidwell-Sterling toluene distillation procedure, and the benzol modification of this method.

Several methods for the determination of "crude" moisture (volatile matter by loss in weight) have been considered without any recommendation for adoption.

Last year Associate Referee Williams reported (*This Journal,* **33,**174) results obtained by the two official wheat flour methods **(20.3** and **20.4)** captioned Total Solids (Moisture, Indirect Method), Vacuum-Oven Method, and Air-Oven Method. The collaborative results on the three types of flour mixes containing sodium bicarbonate were for the most part quite satisfactory for this type of product. It was recommended that the work be continued.

This year ten samples of flour, identified as to type only by a single letter, have been submitted for collaborative study by the Vacuum-Oven Method **(13.3)** and the Air-Oven Method (130°) **(13.4),** with instructions to report a single result on each sample by each of these two methods, taking care to mix the sample well by rolling the jar, with tapping when necessary, before weighing out sample.

* For report of Subcommittee D and action of the Association, see *This Journal,* **34,** 55 (1951).

The collaborators were directed to make certain that an efficacious desiccant is used in the desiccator and in the train through which air passes into the vacuum oven.

It was requested that the report include a description of the ovens used, the desiccants used, and the atmospheric conditions at time of analysis.

The samples sent to each collaborator consisted of duplicate portions from well mixed commercial flour mixes as follows:

Self Rising Flour	marked F&R
Cake Flour	marked A&N
Pancake Mix	marked K&H
Biscuit Mix	marked C&O
Waffle Mix	marked L&S

The non-bicarbonate cake flour was included for comparative purposes.

The various analysts report the use of the following equipment and the following weather conditions.

Analyst 1.—Vacuum oven, Cenco Electric, liquid bath, indirect heat. Air oven, Fisher "Isotemp" gravity convection. Atmospheric conditions: Temperature 80–90°F., relative humidity 36%.

Analyst 2.—Vacuum oven, Weber Electric. Air oven, Cenco, gravity convection. Atmospheric conditions: Cool, cloudy, and damp day.

Analyst 3.—Vacuum oven, Cenco, oil bath electric. Air oven, Elconap ventilated. Atmospheric conditions: Vacuum oven, rainy weather, 60–70°F. Air oven, first weighing during rainy weather, analyzed in clear weather, temperature 80–90°F.

Analyst 4.—Vacuum oven, Cenco, oil bath, electric. Air oven, Freas forced draft. Weather conditions: Cloudy, no rain. Temperature at noon 44°F. Barometric pressure at noon, 29.38, relative humidity 65–73%.

Analyst 5.—Vacuum oven, Cenco, oil jacketed. Air oven, Cenco. Atmospheric conditions (Vacuum oven) Barometric pressure 24.44–24.74 in., Humidity—6. (Air oven) Bar. pressure 24.45–24.72 in. Humidity—10.

Analyst 6.—Vacuum oven, water jacketed, gas heat. Atmospheric conditions: Temperature 23.9–24°C. (75°F.) Humidity 41%–62%. Air oven, Freas force draft. Atmospheric conditions: Temperature 25.1°C. (77°F.) Humidity 41%.

Analyst 7.—Vacuum oven, Cenco, oil bath electric. Air oven, Freas force draft Atmospheric conditions: Weather overcast, Temperature 27° C. (80°F.) Barometric pressure 763.5, relative humidity 85%.

Analyst 8.—Vacuum oven, oil bath, electric. Air oven, Freas force draft. Weather conditions: Sunny in morning, cloudy afternoon with rainfall of .02 in. Max. temperature 57°F. Relative humidity at noon 62%.

The analytical results are tabulated in Table 1. Averages are recorded for each sample, together with maximum and minimum results, and variation between maximum and minimum results for each method.

In Table 2 the average results as calculated by the Associate Referee for duplicate samples are given for each analyst, for each product, together with variation between methods and the grand averages for each product.

TABLE 1.—*Results, per cent moisture, loss in weight, reported by collaborators*
(V.O. = Vacuum-Oven Method, **13.3**; A.O. = Air-Oven Method, **13.4**)

TYPE OF FLOUR		SELF RISING		CAKE FLOUR		PANCAKE MIX		BISCUIT MIX		WAFFLE MIX	
SUB DESIGNATION		F	R	A	N	K	H	C	O	L	S
ANALYST	METHOD										
1	V.O.	11.54	11.51	11.92	11.66	10.26	10.32	9.80	9.70	9.06	9.10
1	A.O.	11.68	11.48	11.34	11.24	10.39	10.16	9.53	9.60	9.06	9.19
2	V.O.	11.32	11.23	11.30	11.40	10.08	10.09	9.55	9.52	8.89	9.05
2	A.O.	11.39	11.33	11.20	11.13	10.44	10.42	9.43	9.54	9.10	9.05
3	V.O.	11.14	11.22	11.08	11.04	10.13	10.22	9.40	9.44	8.77	8.79
3	A.O.	11.10	11.07	11.38	11.29	10.13	10.10	9.47	9.43	8.77	8.69
4	V.O.	11.48	11.44	11.32	11.35	10.18	10.13	9.62	9.62	9.03	8.99
4	A.O.	11.45	11.46	11.38	11.38	10.15	10.17	9.55	9.58	8.86	8.97
5	V.O.	11.49	11.25	11.41	11.15	10.10	10.22	9.60	9.55	8.99	8.93
5	A.O.	11.56	11.56	11.29	10.94	10.24	10.28	9.56	9.67	9.48	9.13
6	V.O.	11.50	11.46	11.54	11.52	10.24	10.25	9.73	9.72	9.10	9.06
6	A.O.	11.48	11.53	11.37	11.40	10.25	10.25	9.67	9.68	9.07	9.04
7	V.O.	11.73	11.71	11.65	11.65	10.29	10.37	9.64	9.61	9.08	9.04
7	A.O.	11.50	11.42	11.29	11.28	10.22	10.19	9.58	9.64	9.02	9.01
8	V.O.	11.23	11.30	11.09	11.14	9.92	9.96	9.43	9.43	8.78	8.92
8	A.O.	11.59	11.77	11.43	11.41	10.47	10.47	9.78	9.78	9.13	9.20
Average	V.O.	11.43	11.39	11.41	11.36	10.15	10.19	9.60	9.57	8.96	8.98
	A.O.	11.47	11.45	11.33	11.26	10.28	10.25	9.57	9.61	9.06	9.03
Max.		11.73	11.71	11.92	11.66	10.29	10.37	9.80	9.72	9.10	9.10
Min.	V.O.	11.14	11.22	11.08	11.04	9.92	9.96	9.40	9.42	8.77	8.79
Var.		.59	.49	.84	.62	.37	.41	.40	.29	.33	.31
Max.		11.68	11.77	11.43	11.41	10.44	10.47	9.78	9.78	9.48	9.20
Min.	A.O.	11.10	11.07	11.20	10.94	10.13	10.10	9.43	9.43	8.77	8.69
Var.		.58	.70	.23	.47	.31	.37	.35	.35	.71	.51

TABLE 2.—*Average* moisture, volatile matter, or loss in weight by drying for flour mixes. Variation in results between methods*
(V.O. = Vacuum-Oven Method **13.3**; A.O. = Air-Oven Method **13.4**; Var. = Variation in Results)

TYPE OF FLOUR		SELF RISING				CAKE FLOUR				PANCAKE MIX				BISCUIT MIX				WAFFLE MIX			
AVERAGE OF SUBS		F & R				A & N				K & H				C & O				L & S			
ANALYST	METHOD	LOSS IN WT. BY		HIGHER % BY		LOSS IN WT. BY		HIGHER % BY		LOSS IN WT. BY		HIGHER % BY		LOSS IN WT. BY		HIGHER % BY		LOSS IN WT. BY		HIGHER % BY	
		V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.	V.O.	A.O.
		Av.		*Var.*		*Av.*		*Var.*		*Av.*		*Var.*		*Av.*		*Var.*		*Av.*		*Var.*	
1	V.O.	11.52				11.79				10.29				9.75				9.08			
	A.O.		11.58				11.29				10.27				9.56				9.12		
	Var.				.04				.50				.02				.19				.04
2	V.O.	11.27				11.35				10.08				9.53				8.97			
	A.O.		11.36				11.17				10.43				9.48				9.07		
	Var.				.09				.18				.35				.05				.10
3	V.O.	11.18				11.06				10.17				9.42				8.78			
	A.O.		11.08				11.33				10.11				9.45				8.73		
	Var.				.10				.27				.06				.03				.05
4	V.O.	11.46				11.33				10.16				9.62				9.01			
	A.O.		11.46				11.38				10.16				9.56				8.91		
	Var.				.00				.05				.00				.06				.10
5	V.O.	11.37				11.28				10.16				9.57				8.96			
	A.O.		11.56				11.11				10.26				9.61				9.30		
	Var.				.19				.17				.10				.04				.34
6	V.O.	11.48				11.53				10.25				9.72				9.08			
	A.O.		11.50				11.385				10.25				9.67				9.05		
	Var.				.02				.15				.00				.05				.03
7	V.O.	11.72				11.65				10.33				9.62				9.06			
	A.O.		11.46				11.28				10.20				9.61				9.01		
	Var.				.26				.37				.13				.01				.05
8	V.O.	11.26				11.11				9.94				9.43				8.85			
	A.O.		11.68				11.42				10.44				9.78				9.16		
	Var.				.42				.31				.50				.35				.31
Av.	V.O.	11.41				11.39				10.17				9.58				8.97			
	A.O.		11.46				11.30				10.27				9.59				9.05		
	Var.				.05				.09				.10				.01				.08

* Averages calculated by Associate Referee from results of analysis of duplicates by collaborators.

DISCUSSION

A review of Table 1 will show that the duplicate samples checked fairly well for each analyst and between analysts for flour type. In most cases there was fairly good agreement between results by the different methods.

Table 2 shows the agreement between the average of 2 results of the analysts. It also shows fairly close agreement between the two methods.

The average of 20 determinations for each flour type by each of two methods shows a variation between the methods from .01% as a minimum

TABLE 3.—*Comparison of "moisture" results on special flours (this report) with collaborative results on ordinary hard and soft wheat flours**

FLOUR TYPE	YEAR	METHOD	
		VACUUM OVEN	AIR OVEN
Tabulation I.—Maximum variation in the results of the individual analyst for each type of flour			
Self Rising	1950	.24%	.20%
Pancake Mix	1950	.12	.23
Biscuit Mix	1950	.10	.11
Waffle Mix	1950	.16	.35
Cake	1950	.26	.35
Hard Wheat	1925	.52	.27
Soft Wheat	1925	.60	.47
Tabulation II.—Maximum variation among the results of different analysts for each type of flour			
Self Rising	1950	.59%	.70%
Pancake Mix	1950	.45	.37
Biscuit Mix	1950	.40	.35
Waffle Mix	1950	.33	.79
Cake	1950	.88	.49
Hard Wheat	1925	.87	.56
Soft Wheat	1925	1.12	.69
Tabulation III.—Maximum variation between the two methods for the grand average moisture content for each type of flour, listed under method giving the higher results			
Self Rising	1950	—	.05
Pancake Mix	1950	—	.10
Biscuit Mix	1950	—	.01
Waffle Mix	1950	—	.08
Cake	1950	.09	—
Hard Wheat	1925	.08	—
Soft Wheat	1925	.11	—

* *This Journal*, 8, 670 (1925).

in the case of biscuit mix to .10% as a maximum variation in the case of pancake mix. It is interesting to note that the non-bicarbonate cake flour shows an average variation of .09% between the methods, with the vacuum method producing the higher result; the other variations between average results by the two methods (.05, .10, .01 and .08) all show the greater moisture content by the air-oven method.

In order to evaluate the significance of those instances of wider variation in the moisture results which were determined by the use of methods official for wheat flour, a comparison is made below (Table 3A) with the original collaborative results which were used as a basis for the official wheat flour methods: See *This Journal*, **8**, 670 (1925).

In the 1925 collaborative work nine analysts were provided with one sample bottle each of hard wheat clear flour and soft wheat straight flour. Each analyst reported four to eight results on each type by each method.

TABLE 3A.—*Comparison of "moisture" results*

FLOUR TYPE	YEAR	METHOD			
		VACUUM OVEN DEVIATION		AIR OVEN DEVIATION	
		ABOVE AV.	BELOW AV.	ABOVE AV.	BELOW AV.
Tabulation IV.—Maximum deviation of individual results of all analysts, from average of results of all analysts for each type of flour, by each method					
Self Rising	1950	.32	.27	.31	.39
Pancake Mix	1950	.20	.25	.20	.17
Biscuit Mix	1950	.22	.18	.19	.16
Waffle Mix	1950	.13	.20	.43	.36
Cake	1950	.53	.35	.13	.36
Hard Wheat	1925	.33	.54	.25	.31
Soft Wheat	1925	.49	.63	.38	.31

It is to be noted that the average moisture (loss in weight) content of the bicarbonate-free flour is greater in each case by the vacuum-oven method, while in the case of the bicarbonate-containing flour the average loss is greater by the air-oven method. This possibly indicates a slightly greater breakdown of the bicarbonate at the higher temperature.

RECOMMENDATIONS*

Since the methods for moisture, Indirect Method, made official for wheat flour, gives concordant results in determining loss in weight for flour mixes containing sodium bicarbonate as an ingredient,

It is recommended—

(1) That vacuum-oven method **13.3** and air-oven method **13.4** be made

* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 54 (1951).

official for moisture (loss in weight by drying), in flour mixes containing sodium bicarbonate as an ingredient, *viz*: Self-rising flour, pancake mix, biscuit mix, and waffle mix.

(2) That this problem be closed.

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REPORT ON ACETIC AND PROPIONIC ACIDS IN BREAD

(MOLD INHIBITORS, PROPIONATES)

By LEWIS H. MCROBERTS (U. S. Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee*

The first report on this subject (1) covered the description of a method for volatile fatty acids in bakery products and collaborative analyses that led to the adoption of a "tentative" procedure (2). The second report (3) had reference to the estimation of losses of acetic and propionic acids during the baking process and during the air drying of bread in preparation for analysis. The following year an alternative method was described (4). This latter procedure was adopted at the 1949 convention with "first action" status and will be included in the 7th edition of *Methods of Analysis, A.O.A.C.* The descriptions of sample preparation together with a rapid distillation procedure are outlined in the Cereal Foods section. The analyst is then referred to the Fish Products section for chromatographic separation and determination of volatile fatty acids (C_1–C_5).

During the present year the Associate Referee has carried out the recommendations of the previous report: (a) to develop a method of sample preparation of fresh bread to insure against spoilage or loss of volatile fatty acids; (b) to submit the method for the determination of acetic and propionic acids described in the 1949 report to collaborative trial.

Detailed instructions were included in the previous report for the distillation of acetic and propionic acids to the point where the chromatographic separation is used. This chromatographic portion of the method has not been received in published form. However, the author was provided with a photostat of the galley proof of the procedure as it will be printed in the Fish Products section of the 7th Ed. *Methods of Analysis*, and from this he completed the method description for the collaborators. These instructions differ from the original in minor respects, *i.e.*, under "Test of silicic acid for suitability . . .," three mixtures of formic, acetic,

and propionic acids have been specified to cover the usual range of acetic and propionic acid in bread with added propionate. The parts that refer to butyric and valeric acids were not included in that they have no reference to the present problem. The essential details have not been changed.

I. SAMPLE PREPARATION

Previous experiments (3) have shown substantial losses of propionic and acetic acids during air drying in the preparation of bread. The method (4) now includes directions for the analysis of fresh bread but does not provide for preserving the prepared sample. This can be accomplished by the addition of chloroform. Finely divided fresh white and wheat breads (ca 10–14 mesh) have been preserved in this manner for periods of 12 to 18 months without any evidence of mold formation. The fresh bread used for the following described collaborative analyses was analyzed by the Associate Referee with and without the addition of chloroform when the sample was first prepared. Additional analyses of the chloroform-treated bread were made about three months later. This corresponds as closely as possible with the time of analysis of the other collaborators and by comparison with the original analysis shows if any changes occurred in the acetic or propionic acid content. The results of these analyses with those determined on the same bread air dried are given in Table 1.

TABLE 1.—*Comparative analyses of one commercial sample of prepared white fresh bread—with and without chloroform preservative, and the same sample prepared by air drying*

DESCRIPTION	ANALYSIS	ACETIC ACID		PROPIONIC ACID	
		THRESHOLD VOLUME-ML.[2]	MG/100 G	THRESHOLD VOLUME-ML.[1]	MG/100 G
	Date	*av.*	*av.*	*av.*	*av.*
Fresh Bread	5/24/50	50, 50 — 50	37.1, 35.8 — 36.5	16, 16 — 16	78.4, 77.6 — 78.0
Fresh Bread +$CHCl_3$	5/24/50	50, 50 — 50	34.5, 34.8 — 34.7	16, 16 — 16	75.1, 74.3 — 74.7
Fresh Bread +$CHCl_3$	8/11/50	48, 49 — 49	38.2, 39.4 — 38.6	15, 15 — 15	72.5, 73.0 — 72.8
Air Dried Bread (Calc. to Fresh Basis)	5/24/50	49, 50 — 50	39.4, 38.8 — 39.1	17, 17 — 17	58.0, 57.6 — 57.8
Air Dried Bread (Calc. to Fresh Basis)	8/11/50	50, 50 — 50	35.8, 35.7 — 35.8	18, 18 — 18	58.7, 58.4 — 58.6

[1] "CB I" Solvent.
[2] "CB I" + "CB 10" Solvent (changed to "CB 10" at 40 ml volume of "CB 1" (Chromatographic mobile solvents of 1% and 10% normal butyl alcohol in $CHCl_3$).

No change in the acetic acid content of the chloroform treated bread was found after storage for three months. Under the same conditions about 4–6% of the propionic acid appears to have been lost. No loss of acetic acid resulted on air drying. However, the propionic acid content was lowered about 20%. This is not in complete accord with the previous study (3) where substantial losses of both acids were brought about by air drying.

II. COLLABORATIVE ANALYSES

One commercial sample of white bread labeled in part ". . . calcium propionate added to retard spoilage. . ." was employed in testing the method. The total sample of 12 loaves was divided into two parts by alternate slices—one-half for preparation by air drying and one-half for fresh sample preparation. The ratio of air dry weight to fresh weight was 0.701. This figure was used to calculate the air dry sample results to the fresh basis. (See Table 1.) The prepared fresh bread was preserved with chloroform as described below.

Ten collaborators were enlisted for the study. Results were received from seven making a total of eight complete reports inclusive of the results of the Associate Referee. They were supplied with one one-pint sample of the prepared fresh bread preserved with chloroform and two one-half pint samples of the same bread air dried. No reference was made to the fact that the two samples of air dried bread were of the same identity. They were each supplied with a copy of the method described below and were requested to report the following data:

(1) The forerun titrations in ml-0.01 *N* for the acid mixtures and samples. (Corrected for "blank titrations.")

(2) The percentage of recovery of acetic and propionic acids from known acid mixtures described in the method.

(3) The threshold volumes determined for formic, acetic, and propionic acids on the basis of the known acid mixtures.

(4) The threshold volumes used as a basis of identification of acetic and propionic acids (Fresh and Air Dried Bread).

(5) Duplicate determinations of acetic and propionic acids in mg./100 g. of the samples as received.

METHODS

(Acetic and Propionic Acids in Bakery Products)

I. PREPARATION OF SAMPLE

(*a*) *Air-dried bread.*—For analysis of the air-dried product prepare sample as directed in section **20.83,** 6th Ed., *Methods of Analysis.*

(*b*) *Fresh bread or cake.*—For analysis of the fresh product which may be difficult to air dry without spoilage or loss of volatile fatty acids, pass the sample thru a meat grinder equipped with a $\frac{1}{8}$" hole plate and reduce to finely divided condition by rubbing through an 8-mesh sieve. Proceed with the analysis promptly (24–48 hrs.) or preserve with chloroform.

(c) *Fresh bread or cake—Preserved with chloroform.*—To the bread prepared as in (b) in a Mason jar filled to ¾ capacity, add washed chloroform adsorbed in ca 1 g of cotton (ca 5 ml $CHCl_3$ per pint of container size). Close the jar tightly (self-sealing lids are recommended), and mix the contents thoroly by rolling. Samples may be stored at temperatures of ca 25°C. Refrigeration is recommended where higher temperatures obtain.

II. REAGENTS

(1) *Silicic acid.*—Reagent grade.[1]

(2) *Indicators:*

(a) *Alphamine Red R.*—(Also referred to as "RNH_4 indicator"); 0.2% in water.

(b) *Cresol Red.*—Dissolve 50 mg in 20 ml of ethyl alcohol, add 1.3 ml of 0.1 *N* NaOH and make to 50 ml with water.

(c) *Phenolphthalein.*—1% in 95% ethyl alcohol.

(3) *Solvents:*

(a) *1% n-butanol-chloroform.*—Remove ethyl alcohol from U.S.P. chloroform by washing it three times with distilled water, using a quantity of water equal to about ½ the volume of chloroform. To one liter of washed chloroform in a separatory funnel add 10 ml of *n*-butanol (acid free) and shake vigorously. Add 25 ml of water and shake again. Allow chloroform to stand until clear, draw off and discard water. (Referred to as "CB-1.")

(b) *10% n-butanol chloroform.*—Mix 900 ml U.S.P. chloroform, 100 ml *n*-butanol and 25 ml water in a separatory funnel. Shake vigorously and separate from water. (Do not wash chloroform to remove ethyl alcohol.) (Referred to as "CB 10.")

(4) *Alkali solutions:*

(a) *NaOH.*—ca 1 *N*.

(b) *NH_4OH.*—ca 1 *N*.

(c) *$Ba(OH)_2$ 0.01 normal.*—Store in a paraffin lined bottle protected from CO_2 by soda-lime absorbent. Dispense from a 10 ml burette.

(5) *Acids:*

(a) *H_2SO_4 (1+1) or H_3PO_4.*—85%.

(b) *Formic, acetic, and propionic, acids of reagent grade.*

(6) *Miscellaneous:*

(a) *Sodium sulphate* (anh.).

III. APPARATUS

(a) *Distillation app.*—The Hillig volatile acid apparatus (*Methods of Analysis*, 6th Ed., 24.10, Fig. 37) can be used to good advantage for the present purpose. Use 150-ml distillation flask and so adjust the voltage (ca 80) that 200 ml is distilled in 35–40 min. An ordinary gas-fired steam generator may also be used.

(b) *Chromatographic tube* (ca 15×250 mm) constricted at lower end to ca 4 mm inside diam.

(c) *Test tubes*, glass stoppered.—(ca 16×150 mm).

(d) *Eyedropper pipette.*—(sufficient length to transfer solvent from test tubes to top of silicic acid column).

IV. DETERMINATION

Distillation:

Transfer 10 g of the air-dried bread or 15 g of the fresh bread to 150 ml distilling flask. Add 50 ml of H_2O and 10 ml of ca 1 *N* H_2SO_4. Mix thoroly and add 10 ml of

[1] Mallincrodt's "Acid Silicic for Chromatography $SiO_2 \times H_2O$ Analytical Reagent Water-Max 20%" was found to be satisfactory. The Associate Referee used 1.5 ml H_2O per 5 g of the acid.

20% phosphotungstic acid soln. Mix by swirling and add 40 g of $MgSO_4 7H_2O$. Swirl again to partially dissolve salt. The mixture should now be acid to Congo Red paper; if not, acidify with H_2SO_4 (1+1). Connect to condenser and steam generator, heat to boiling, and distill 200 ml in 35–40 min.[2] Maintain a volume of ca 60–80 ml in the distilling flask by means of a small burner. Transfer the distillate to a 400–600 ml beaker, add ca 10 ml of 0.01 *N* formic acid,[3] make alkaline to phenolphthalein with ca 1 *N* NaOH, and evaporate to ca 5 ml. Transfer to a g.s. test tube of 25–30 ml capacity, rinsing beaker with three 5 ml portions of H_2O. If insoluble material adheres, add a few drops of ca 1 *N* H_2SO_4 with one rinse. Make alkaline to phenolphthalein and evaporate just to dryness by inserting the tube in a steam bath or in boiling water (air jet hastens evap.). Determine acetic and propionic acids by the following described chromatographic procedure.

CHROMATOGRAPHIC SEPARATION

(*a*) *Preparation of partition column.*

To ca 5 g of silicic acid in a mortar add 1 ml of "RNH_4" indicator soln and sufficient ca 1 *N* NH_4OH to give the alkaline color of the indicator (1–2 drops are usually sufficient). Add the maximum quantity of H_2O that the silicic acid will hold without becoming sticky or agglomerating in the butanol-$CHCl_3$ soln. (This quantity must be determined for each batch of silicic acid and usually varies from 50–75% of wt. of silicic acid.) Mix thoroly with pestle until homogeneous. Add a few ml of the 1% butanol-$CHCl_3$; mix to form a paste, and then add sufficient solvent (ca 25 ml) to form a slurry that will pour readily. Pour this slurry into the chromatographic tube containing a small plug of cotton in the constructed end. To avoid air pockets, tilt the tube slightly while pouring. If air bubbles do form, eliminate by stirring suspension in tube with a long glass rod.

Clamp tube in ring stand in vertical position. In top insert 1-holed rubber stopper fitted with glass tube bent to 90° angle and held in place by Bunsen clamp. Connect glass tube to pressure source and adjust pressure to 5–10 lb/sq in so that excess solvent is forced thru column dropwise.

During removal of excess solvent the gel will pack down. After gel can no longer be poured, more excess solvent can be forced from the column. As column packs down particles of gel will adhere to walls of the tube, but eventually gel will leave walls of tube relatively clean. At this point optimum density for column has been reached, and column is ready for use. Do not allow column to dry below surface of the gel as such drying or "cracking" renders column useless. (If column cracks before acids have been added, gel can be extruded from tube, reslurried with solvent, and again poured into tube. If gel is not packed evenly or if air pockets are present, jagged fronts may occur where soln passes such points of separation.)

(*b-1*) *Test of silicic acid for suitability and standardization of column.*

Prepare stock solns of formic, acetic, and propionic acids by diluting 5 ml to 250 ml and standardize the acetic and propionic acids as follows: Pipette 1 ml into a 125-ml Erlenmeyer flask; dilute to ca 15 ml with boiled water and titrate with 0.01 $Ba(OH)_2$ using cresol red indicator. End point is reached when soln assumes pink color that persists for ca 45 seconds. During course of the titration bubble stream of CO_2 free air or N thru soln.

Prepare the following dilutions from the stock solns with boiled water:

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[2] The steam source may be connected during the heating to boiling. This will prevent the steam tube from becoming clogged with bread solids.

[3] The small amount of formic acid normally present in bread is not sufficient to provide a distinct band in the chromatographic column that can be moved down to a definite threshold. The addition of ca 1 ml. 0.1 *N* formic acid provides a band that can be moved to this point—thus insuring the complete elution of the preceding acetic acid.

Formic acid—10 ml to 50 ml.
Acetic acid—20 ml to 50 ml.
Prepare the following mixtures of formic acid and known amounts of acetic and propionic acids:

"A" Formic acid—10 ml stock solution
Acetic acid—10 ml stock solution
Propionic acid—10 ml stock solution
H_2O—20 ml.
Total of 50 ml of which take 1 ml for separation check.

"B" Formic acid—10 ml stock solution
Acetic—10 ml stock solution
Propionic—30 ml stock solution
Total of 50 ml of which take 1 ml for separation check

"C" Formic acid—1 ml diluted stock solution
Acetic—1 ml diluted stock solution
Propionic—1 ml stock solution
Total of 3 ml for separation check.

(The above mixtures are designed to cover the range of acetic and propionic acids usually present when 15 g of fresh bread, preserved with propionate, is used as the initial sample.)

Pipette the above indicated aliquot of acids into the bottom of g.s. test tubes (ca 16×150 mm), neutralize with ca normal NaOH, using phenolphthalein indicator, and add 1 drop of NaOH soln in excess. Place test tube in steam bath or boiling H_2O bath and allow soln to evaporate to dryness. (Process can be speeded by carefully playing a stream of air on soln, or by carefully inserting vacuum line to a point a few cm above surface of soln; it is essential that none of the salts be lost during this process, spattering must be avoided.)

(b-2) Separation technique

When soln has evaporated to dryness add 2 ml of the 1% butanol in $CHCl_3$ to the test tube and then add 2 drops of $H_2SO_4(1+1)$. Stopper tube immediately and shake gently until all Na salts are converted to free acids.[4] Take care to convert any of salts that may have been deposited on sides of tube without wetting stopper. (Too much H_2O will be indicated by several large drops of insoluble liquid in butanol-$CHCl_3$ mixture; this H_2O may be removed by the addition of a few mg of anh. Na_2SO_4; or if only a few small droplets of insoluble liquid are present, they may be eliminated by rotating tilted test tube and shaking gently until insoluble liquid adheres to the glass walls.)

Place a 50 ml graduated cylinder under the prepared column to catch the forerun, and transfer the butanol-$CHCl_3$ extract of fatty acids to the top of the column with the eye-dropper pipette. Exercise care to avoid disturbing the surface of the gel. (If the surface of the gel is dented or splashed by careless addition of solvent, uneven band fronts will result which will make the accurate collection of each band difficult.) Allow soln to sink into gel under pressure. Rinse test tube with three ml portions of the 1% butanol in $CHCl_3$ and transfer the washings to column allowing each to sink into gel before next is added. After last washing has sunk into gel, fill tube with the 1% butanol in $CHCl_3$ and renew pressure. Change receiver (50 ml graduated cylinder) each time the lower edge of a band reaches a point ca 2–5 mm above the cotton plug in the constricted end of the tube. Propionic acid will elute first followed by acetic acid. At a definite total volume of 1% butanol-chloroform eluate (5) and previous to the threshold of acetic acid[5] release the pressure, pour off

[4] The addition of several glass beads will aid in bringing the solids into contact with the sulphuric acid and solvent.

[5] Recent experiments have shown that the threshold volume—the amount of mobile solvent required to move each acid from the top of the column to the point of emergence—is a function of the concentration of the acid. In the amounts of formic and acetic acids in bread, the differences are not critical. However, the threshold volume of propionic may vary as much as 100%. Therefore to use threshold volume as a means of identification it becomes necessary to determine what the threshold should be for the amount determined.

and discard any solvent above the column and fill the tube with the 10% butanol chloroform. Proceed with the elution of the acetic acid band to the threshold of formic acid.

Record the volumes of the forerun and the volumes in each eluate receiver and calculate threshold volumes. With respect to the trial mixtures and in the usual bread analysis where propionate has been added only three bands will appear on the column. In this event the forerun is the threshold volume for propionic acid and the forerun plus the volume required to elute the propionic acid is the threshold volume for acetic acid.

Transfer the forerun[6] and the eluates to 125 ml Erhlenmeyer flasks, rinsing the graduated cylinder in each case with three 5-ml portions of boiled H_2O. Add 1 drop of the cresol red indicator and titrate with the 0.01 *N* alkali in CO_2-free atmosphere as described previously. However, as end point is approached, stopper flask and shake vigorously to extract acids completely from the solvent phase.

Correct titrations for a blank determined as follows: Collect 25 ml of the butanol-$CNCl_3$ mixture from the column before any acids are transferred, add 15 ml of boiled H_2O and titrate as above with the 0.01 *N* alkali.

(*c*) *Identification and Determination*

Proceed as described above under (*b-2*) on the basis of the evaporated distillates.

Identify the acids by comparing their threshold volumes with those for ca same quantities of known acids used in standardization procedure. However, if conditions are changed, such as by use of different batch of silicic acid or different quantity of same batch or different quantity of H_2O, the threshold volume of each acid must be redetermined.

The separated acids may be further identified by means of the formation of mercurous acetate and mercurous propionate crystals (6).

Calculate the results for acetic and propionic acids to mg/100 g. The following factors are based on 15 g of fresh bread and 10 g of air-dried bread.

Fresh Bread	Acetic	4.00×ml	0.01 Normal
	Propionic	4.93×ml	0.01 Normal
Air-Dried Bread	Acetic	6.00×ml	0.01 Normal
	Propionic	7.40×ml	0.01 Normal

COLLABORATOR COMMENTS

Frank J. McNall:

"No correction was made for blank titration since the blank consumed less than one drop of N/100 $Ba(OH)_2$. I do not like the strength of the RNH_4 indicator called for in the method since it gives a color which is too dark when made up fresh. Instead of using 1 ml as directed in the method, I found that 8 drops of indicator plus 1 drop of *N* NH_4OH and 1.65 ml of H_2O gave the best column to work with. If more than 1 drop of NH_4OH is used the recovery of the acids was lowered appreciably.

"I prefer the use of 25-ml beakers in place of test tubes for the final evaporation

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The separation of the mixtures "A", "B", and "C" should supply sufficient data for the present purpose. It is suggested that threshold volumes be plotted against the titrations. Thresholds can then be predicted for intervening concentrations. After development of the bands with the "CB-1" solvent, the elution may be speeded by changing to the "CB-10" solvent. Due to the possibility of differences in the propionic threshold, this change should be made at a constant "CB-1" eluate volume rather than at the propionic threshold (5), preferably when the greater part of the propionic is eluted. By this means the acetic threshold will not be affected by changes in the propionic threshold. The Associate Referee used 40 ml of "CB-1" as the point of change to "CB-10."

[6] The titration of the forerun previous to the propionic threshold in the pure acid mixtures should be the equivalent of the "blank titration." However, in bread analyses, the forerun previous to propionic may titrate higher than the blank. This may be due to traces of higher acids.

of the distillate. They are much handier to use and the evaporation takes place at a more rapid rate."

L. C. Mitchell:

"The instructions were adhered to with the following exceptions:

"Used 250-ml distilling flask instead of 150 cc as specified, which probably is a typographical error.

"Evaporated the distillate in 400 ml beakers to 1–2 ml, then transferred the soln to test tube with eyedropper pipet and rinsed pipet and beaker with 1-ml portions of water holding the total volume to under 10 ml.

"Instead of using the grinding procedure to prepare the silica column, it was prepared as follows:

"Place 5 g silicic acid into a dry pear-shape 125 ml separatory funnel, add 25 ml of CB 1 solvent, shake thoroly, add 1.0 ml indicator soln, 1.5 ml water and 1 drop N/1 NH_4OH, shake vigorously until mixture is homogeneous, and transfer slurry formed to chromatographic tube.

"In my hands the 'shaking' procedure gives a homogeneous column; something I seldom was certain of when using the 'grinding' operation. 40 ml CB 1 solvent was used in accordance with note 6, page 290, before changing to solvent CB 10. This was done by adding a total volume of 40 ml and checking against volume of percolate."

H. M. Bollinger:

"When the distillate is transferred from the receiver to a beaker, the fatty residue is difficult to remove even with hot water. I added 3 drops of 1 *N* NaOH to the last rinse.

"I find that release of the organic acids into CB 1 may be performed in an ordinary test tube, without glass beads, by using a slightly curved pointed stirring rod for agitation and scratching. The CB 1 is then decanted down the stirring rod into the partition column and the lip of the test tube is washed with a few drops of solvent. It seems easier for me than trying to police both tube and glass stopper without errors."

H. O. Fallscheer:

"The method is quite straightforward, and I experienced no particular difficulties. You have specified a 150 ml distillation flask, which is not a common size. The 125 ml size seemed too small, and I settled on a 200 ml size. I presume the flask size within limits does not materially affect the results. Your statement 'Maintain a volume of 60–80 cc in the distillation flask' might be a little confusing since you start out with a volume of about 100 ml. I ended up with a final volume of 70–80 ml."

DISCUSSION

The results of analysis of the standardized mixtures of pure acids are tabulated in Table 2. The descriptions given under the headings of Mixtures A, B, and C were obtained from the standardizations of the acids used by the Associate Referee. The other collaborators would have slightly different mixtures, depending on the purity of the concentrated acids used for the specified dilutions. The percentages recovered demonstrate that each collaborator was able to make efficient separations of the three acids and accurate determinations of acetic and propionic acids. Considerable variation is found in threshold volumes. However, this would be expected

from the use of different lots of silicic acid and variations in column preparation. The analyst must keep his procedure uniform and is thus able to identify acids by the threshold volumes that he has determined. The threshold volume for propionic acid was found to vary as much as 100% over the range of amounts that are usually found in bread with added propionate. Therefore, it is necessary to have sufficient data to predict what the threshold volume should be for a given concentration. This may be accomplished by plotting threshold volumes against ml of 0.01 *N* acid. Where three bands are developed on the column the lowest band will usually be propionic acid. Two bands are indicative of only formic and acetic acids.

The results of analysis of the bread samples are tabulated in Table 3. In general they are considered to be in good agreement. The amounts of acetic and propionic acid determined are found to be close to those specified in the pure acid "Mixture B" of Table 2. The threshold volumes determined for that mixture should therefore be in close agreement with those volumes found during the separation of the acids from the bread. On the basis of the average threshold volumes, these figures agree within 2 ml. or better in about 90% of the determinations—sufficiently close for the identification.

TABLE 2.—*Chromatographic separation of known amounts of acetic and propionic acids*

(Test of chromatographic column mixtures)

	MIXTURE "A"[8]							
	FORMIC 9.40 ML N/100 OR 4.33 MGS.[7] ACETIC 6.88 ML N/100 OR 4.14 MGS. PROPIONIC 5.06 ML N/100 OR 3.74 MGS.							
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$ (Ave.)	0.10	0.05	0.03	0.05	—	0.0	0.0	0.0
Propionic Acid Recovery %	99.5 99.5	97.9 —	96.9 96.9	100.2 94.7	99.0 —	98.5 —	97.0 —	99.7 105.4
(Ave.)	99.5	97.9	96.9	97.5	99.0	98.5	97.0	102.6
Propionic Acid Threshold Vol. (ml CB 1)[1]	24 23	18 —	21 20	20 15	25 —	22 —	15 —	
(Ave.)	24	18	21	18	25	22	15	
Acetic Acid Recovery %	103.2 102.8	100.3 —	99.7 99.0	92.8 94.9	99.0 —	97.0 —	96.2 —	95.1 89.4
(Ave.)	103.0	100.3	99.4	93.9	99.0	97.0	96.2	92.3
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	51 —	48 —	49 49	51 46	57 —	59 —	45 —	62 61
(Ave.)	51	48	49	49	57	59	45	62
Formic Acid Threshold Vol. (ml CB 1+CB 10)[2]	67 —	63 —	62 61	66 60	74 —	75 —	57 —	
(Ave.)	67	63	62	63	74	75	57	

[7] ca 1 ml 0.1 *N* Formic Acid as an aid in Acetic Acid Separation.
[8] Test mixtures standardized by Associate Referee.

TABLE 2.—(*Continued*)

	MIXTURE "B"[8]							
	FORMIC 9.40 ML N/100 OR 4.33 MGS.[7] ACETIC 6.88 ML N/100 OR 4.14 MGS. PROPIONIC 15.20 ML N/100 OR 11.25 MGS.							
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$ (Ave.)	0.10	0.70	0.03	—	—	0.0	0.0	0.35
Propionic Acid Recovery %	98.5 97.8	97.5 —	97.9 97.6	98.9 95.0	96.5 —	97.6 —	98.7 —	88.8 87.2
(Ave.)	98.2	97.5	97.8	97.0	96.5	97.6	98.7	88.0
Propionic Acid Threshold Vol. (ml CB 1)[1]	16 15	13 —	15 15	17 14	13 —	15 —	13 —	15 15
(Ave.)	16	13	15	16	13	15	13	15
Acetic Acid Recovery %	101.5 101.5	92.3 —	101.2 101.2	98.7 99.2	99.0 —	98.4 —	96.8 —	115.7 100.0
(Ave.)	101.5	92.3	101.2	99.0	99.0	98.4	96.8	107.9
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	49 —	48 —	48 49	51 50	54 —	59 —	47 —	57 53
(Ave.)	49	48	49	51	54	59	47	55
Formic Acid Threshold Vol. (ml CB 1+CB 10)[2]	65 —	63 —	60 62	68 68	69 —	73 —	60 —	
(Ave.)	65	63	61	68	69	73	60	

TABLE 2.—(*Continued*)

	MIXTURE "C"[8]							
	FORMIC 9.40 ML N/100 OR 4.33 MGS.[7] ACETIC 13.80 ML N/100 OR 8.27 MGS. PROPIONIC 25.30 ML N/100 OR 18.70 MGS.							
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$ (Ave.)	—	0.05	0.03	—	—	0.0	—	0.02
Propionic Acid Recovery %	97.5 98.4	96.3 —	97.1 97.7	98.8 99.9	97.6 —	97.7 —	98.7 —	97.8 100.1
(Ave.)	98.0	96.3	97.4	99.4	97.6	97.7	97.8	99.0
Propionic Acid Threshold Vol. (ml CB 1)[1]	13 13	11 —	13 13	12 15	16 —	13 —	11 —	11 9
(Ave.)	13	11	13	14	16	13	11	10
Acetic Acid Recovery %	93.6 96.3	100.6 —	101.6 100.8	99.5 100.7	96.1 —	97.8 —	98.7 —	92.7 99.3
(Ave.)	95.0	100.6	101.2	100.1	96.1	97.8	98.7	96.0
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	49 —	47 —	48 48	47 51	56 —	57 —	47 —	53 50
(Ave.)	49	47	48	49	56	57	47	52
Formic Acid Threshold Vol. (ml CB 1+CB 10)[2]	65 —	63 —	62 62	65 60	73 —	75 —	62 —	
(Ave.)	65	63	62	63	73	75	62	

TABLE 3.—*Acetic and propionic acids in commercial white bread*
(Collaborative Analyses—Basis One Sample—Prepared Fresh and Air Dried)

	FRESH BREAD (PRESERVED WITH $CHCl_3$)								
	SUB NO. 1 (FRESH BASIS)								
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)	(Av.)
Propionic Acid (mg/100 g)	75.1 74.3 72.8	74.1 72.3 —	77.6 77.4 —	53.6 71.0 67.0	59.7 61.3 —	77.0 76.2 —	75.2 73.2 —	75.5 74.4 —	
(Ave.)	74.1	73.2	77.5	63.9	60.5	76.6	74.2	75.0	71.9
Propionic Acid Threshold Vol. (ml CB1)[1]	16 16 15	13 — —	15 16 —	22 14 15	24 25 —	15 16 —	14 13 —	16 18 —	
(Ave.)	16	13	16	17	25	16	14	17	17
Acetic Acid (mg/100 g)	34.5 34.8 38.8	34.9 33.2 —	41.6 41.4 —	37.4 42.9 37.3	32.0 36.0 —	36.3 36.2 —	40.1 39.3 —	38.0 33.8 —	
(Ave.)	36.0	34.1	41.5	39.2	34.0	36.3	39.7	35.9	37.1
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	50 50 49	48 — 48	48 49 49	52 49 —	56 58 —	59 58 —	47 47 —	61 61 —	
(Ave.)	50	48	49	51	57	59	47	61	—
Formic Acid-Threshold Vol. (ml CB 1+CB 10)[2]	67 66 66	63 — —	60 62 —	70 66 67	72 75 —	75 74 —	— — —	73 —	
(Ave.)	66	63	61	68	74	75	—	—	—
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$)	0.54 0.43 0.55	0.38 0.28 —	0.40 0.40 —	0.40 0.50 0.60	— — —	0.43 0.43 —	0.40 0.36 —	0.29 0.15 —	
(Ave.)	0.51	0.33	0.40	0.50	—	0.43	0.38	0.22	0.40

	AIR DRIED BREAD								
	SUB NO. 2 (AIR DRY BASIS)								
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)	(Av.)
Propionic Acid (mg/100 g)	83.8 81.8 84.4	87.5 87.2 —	90.3 89.9 —	63.4 66.6 —	80.8 80.4 —	86.7 88.1 —	87.4 88.9 —	88.9 88.2 —	
(Ave.)	83.3	87.4	90.1	65.0	80.6	87.4	88.2	88.6	83.8
Propionic Acid Threshold Vol. (ml CB 1)[1]	17 16 18	13 — —	16 17 —	16 16 —	23 18 —	17 17 —	14 13 —	24 19 —	
(Ave.)	17	13	17	16	21	17	14	22	17
Acetic Acid (mg/100 g)	51.3 — —	39.3 41.3 —	47.4 45.9 —	39.3 41.7 —	40.5 41.4 —	42.3 43.4 —	44.4 44.3 —	32.6 34.9 —	
(Ave.)	51.3	40.3	46.7	40.5	41.0	42.9	44.4	33.8	42.6
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	49 49 50	48 — 48	49 49 49	48 50 —	56 55 —	59 59 —	48 45 —	62 58 —	
(Ave.)	49	48	49	49	56	59	47	60	—
Formic Acid-Threshold Vol. (ml CB 1+CB 10)[2]	66 65 68	63 — —	62 62 —	64 68 —	72 73 —	74 74 —	— — —	72 69 —	
(Ave.)	66	63	62	66	73	74	—	71	—
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$)	0.54 0.54 0.46	0.33 0.33 —	0.35 0.35 —	0.60 0.60 —	— — —	0.34 0.37 —	0.30 0.33 —	0.31 0.25 —	
(Ave.)	0.51	0.33	0.35	0.60	—	0.36	0.32	0.28	0.39

TABLE 3.—(*Continued*)

	AIR DRIED BREAD								
	SUB NO. 3 (AIR DRY BASIS)								
Collaborator No. Footnote No.	1 (3)	2 (3)	3 (3)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)	(Av.)
Propionic Acid (mg/100 g)	83.8 80.7 84.8	83.0 85.8 —	89.5 89.9 —	73.4 85.0 —	79.6 85.0 —	89.0 88.9 —	89.1 87.6 —	87.7 84.5 —	
(Ave.)	83.1	84.4	89.7	79.2	82.3	89.0	88.4	86.1	85.3
Propionic Acid Threshold Vol. (ml CB 1)[1]	18 16 18	16 — —	17 16 —	15 15 —	17 15 —	17 16 —	14 15 —	14 28 —	
(Ave.)	17	16	17	15	16	17	15	21	17
Acetic Acid (mg/100 g)	51.0 — —	38.8 40.7 —	48.3 45.3 —	45.3 51.6 —	45.6 50.7 —	44.2 44.5 —	43.4 43.4 —	40.1 38.4 —	
(Ave.)	51.0	39.8	46.8	48.5	48.1	44.4	43.4	39.3	45.2
Acetic Acid Threshold Vol. (ml CB 1+CB 10)[2]	50 50 50	48 — —	49 49 —	48 49 —	49 51 —	59 59 —	47 48 —	51 67 —	
(Ave.)	50	48	49	49	50	59	48	59	—
Formic Acid-Threshold Vol. (ml CB 1+CB 10)[2]	67 67 68	62 — —	62 61 —	63 66 —	65 65 —	75 74 —	— — —	67 — —	
(Ave.)	67	62	62	65	65	75	—	—	—
Forerun Titration (ml 0.01 *N* $Ba(OH)_2$)	0.48 0.48 0.52	0.28 0.33 —	0.35 0.35 —	0.50 0.50 —	— — —	0.38 0.39 —	0.33 0.30 —	0.50 0.12 —	
(Ave.)	0.49	0.31	0.35	0.50	—	0.39	0.32	0.31	0.38

[1] CB 1 (1% normal butyl alcohol in $CHCl_3$).
[2] CB 10 (10% normal butyl alcohol in $CHCl_3$).
[3] Changed from CB 1 to CB 10 at 40 ml CB 1.
[4] Changed from CB 1 to CB 10 at 45 ml CB 1.
[5] Changed from CB 1 to CB 10 at 50 ml CB 1.

CONCLUSIONS

It has been demonstrated that the above described method is adequate for the determination and identification of both acetic and propionic acids in bread. Previous investigations have shown that propionic acid has not been a normal ingredient in bread and therefore its presence is indicative of added propionate. The presence of acetic acid is normal and in amounts that must be taken into account in the detection of added acetic acid whether from vinegar or "sodium diacetate."

Fresh bread sample preparation should be used to give the most accurate determinations of the acetic and propionic acid content.

Prepared fresh bread samples may be adequately preserved by the addition of chloroform.

Air drying in sample preparation generally results in substantial losses of acetic and propionic acids. However, the loss of propionic acid is not critical where the only purpose of the analyst is the detection of added propionate.

RECOMMENDATIONS*

It is recommended—

That this refereeship be continued for the following described purposes:

(A) For review of the method for volatile fatty acids adopted as first action (7) that will be included in the 7th ed., *Methods of Analysis*,

(B) For further consideration of the procedure outlined in this report relative to any changes that may be necessary in the method described in (A).

ACKNOWLEDGMENT

The Associate Referee acknowledges with appreciation the assistance of the following collaborators from the Food and Drug Administration:

Frank J. McNall, Cincinnati District; L. C. Mitchell, Minneapolis District; V. E. Munsey, and Arnold Lada, Washington, D. C.; Howard M. Bollinger, Los Angeles District; H. O. Fallscheer, Seattle District; Hugh M. Boggs, Philadelphia District.

REFERENCES

(1) McRoberts, Lewis H., *This Journal*, **31**, 489 (1948).
(2) ——, *Ibid.*, **31**, 99 (1948).
(3) ——, *Ibid.*, **32**, 496 (1949).
(4) ——, *Ibid.*, **33**, 677 (1950).
(5) Hillig, Fred, Personal Communication (1950).
(6) Patterson, W. I., and Ramsey, L. L., *This Journal*, **28**, 644 (1945).
(7) *Changes in Methods of Analysis*, *Ibid.*, **33**, 86 (1950).

REPORT ON BAKING POWDERS

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

The neutralizing value of anhydrous monocalcium phosphate, monocalcium phosphate monohydrate, and sodium acid pyrophosphate has been determined by 5 analysts. The method in Sec. 7.8, p. 119, was used on the monohydrate and a slight variation of it on the anhydrous monocalcium phosphate. The method, sec. 7.9, slightly modified, was used on the sodium acid pyrophosphate.

The results are given in Table 1.

The true neutralizing value for these acid salts is not known. The maximum deviation from the average for the 5 analysts reporting represent not over 4% of the neutralizing values, which would seem to be within the limits of acceptable precision.

The qualitative test for Al under Sec. **7.19**, p. 121, sometimes gives results of indefinite character on samples of baking powder known to con-

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 54 (1950).

TABLE 1.—*Neutralizing value*

COLLABORATOR	MONOCALCIUM PHOSPHATE MONOHYDRATE	ANHYDROUS MONOCALCIUM PHOSPHATE	SODIUM ACID PYROPHOSPHATE
		Parts of $NaHCO_3$ per 100 parts of phosphate	
1	84.7	88.2	71.4
	84.7	88.1	71.5
	Av. 84.7	88.1	71.5
2	83.4	84.0	71.4
	83.4	84.0	71.8
	83.1	84.1	71.5
	Av. 83.3	84.0	71.6
3	83.8	84.0	73.0
	83.4	84.0	72.6
	Av. 83.6	84.0	72.8
4	79.9	82.0	69.1
	80.1	82.0	69.0
	Av. 80.0	82.0	69.1
5	81.6	86.5	72.3
	81.7	85.1	71.9
	Av. 81.7	85.8	72.1
6*	81.6, 80.6 } or 81.1	84.0, 83.0 } or 83.5	73.0, 71.4 } or 72.2
Max.	84.7	88.1	72.8
Min.	80.0	82.0	69.1
Av.	82.7	84.8	71.4

* Too late for inclusion in average.

tain aluminum salts. A method designated Method II, suggested by Peter Novitsky of General Foods Corp. for Al, was compared with the A.O.A.C. procedure, Sec. **7.19**, designated Method I. Details of these methods are published in *This Journal*, **34**, 60 (1951).

COLLABORATORS	
1	Prefers Method II
2	Prefers Method II
6	Prefers Method II
4	Prefers Method II
5	Prefers Method I
6	No choice

Method II is preferred by 4 of the 5 analysts.

The assistance of the following collaborators is gratefully acknowledged: Dick Ludwig, Standard Brands, Inc., Chicago 9, Ill.; Peter Novitsky,

General Food Corp., Chicago 24, Ill.; Colin C. Gorton, The Rumford Co., Rumford 16, R. I.; William T. Martin, K. C Foods Division, North Little Rock, Ark.; E. A. Vaupel, R. B. Davis Co., Hoboken N. J.

RECOMMENDATIONS*

It is recommended—

(1) That the methods for anhydrous monocalcium phosphate, nonocalcium phosphate monohydrate, and sodium acid pyrophosphate be adopted, first action, as given in this report, to replace secs. **7.8** and **7.9.**

(2) That the qualitative test, Method II, be adopted, first action, to replace secs. **7.18** and **7.19.**

REPORT ON COSMETICS

By G. ROBERT CLARK (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Associate Referee on Deodorants and Anti-Perspirants has recommended the adoption, first action, of methods for urea, chlorides, and sulfates in deodorants, and that the topic be continued. The Referee concurs in this recommendation.†

The Referee also recommends continuance of the topics:

Cosmetic Creams
Cosmetic Skin Lotions
Mascara, Eyebrow Pencil, and Eyeshadow
Sun Tan Lotion
Hair Dyes and Rinses

The Referee further recommends that the topic *Cosmetic Powders* be discontinued.

No formal report was submitted on mascaras, eyebrow pencils, and eye shadows.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 54 (1951).
† For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 46 (1951).

REPORT ON DEODORANTS AND ANTI-PERSPIRANTS

By HENRY KRAMER (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

DETERMINATION OF UREA IN DEODORANTS

Several methods for the determination of urea in deodorants were investigated. Total nitrogen by semi-micro Kjeldahl (1) was tried and discarded because of its non-specificity. Marshall's method (2) based on the urease enzymatic hydrolysis of urea to ammonia gave excellent results with aqueous urea solutions, but the common deodorant ingredients, zinc and boric acid, interfered. The proposal of Benedict (3) to convert urea into ammonia by heating with potassium acid sulfate and zinc sulfate at 162°C. failed to give good results in the presence of glycerine. Folin's method (4), little subject to interferences, was found to be the most suitable for the determination of urea in deodorants. In principle the urea is hydrolyzed to ammonia by refluxing with magnesium chloride hexahydrate and concentrated hydrochloric acid. The ammonia is then distilled from alkaline solution and titrated in the usual manner. Ammonium salts, if present, will interfere. This laboratory has not, however, found any interfering substances in commercial samples examined.

METHOD

REAGENTS

Magnesium chloride hexahydrate, C.P.
Hydrochloric acid, concentrated.
Sodium hydroxide, 0.1 *N*.
Sulfuric acid, 0.1 *N*.
Methyl red indicator soln.

DETERMINATION

Pipet an aliquot containing 50–100 mg of urea into a 100-ml round-bottom flask having a ST 24/40 female joint. Acidify with concentrated HCl using 0.5 ml in excess; immerse flask in steam bath and evaporate to dryness. Add 10 g of crystalline $MgCl_2.6H_2O$ and 1 ml of concentrated HCl, and connect the flask to a reflux condenser. Heat the mixture carefully with a small flame[1] until the magnesium chloride dissolves in its water of crystallization, and reflux slowly for 2 hours so that the rate of return of liquid from the condenser is 9–14 drops per min. Allow the soln to cool, add water thru the top of the condenser, disconnect the flask, and if necessary heat to dissolve any solids. Transfer the soln to a 1-liter flat-bottom flask, dilute to ca 400 ml with water, make alkaline with 10% NaOH, and distill ca 275–300 ml into a suitable portion of 0.1 *N* H_2SO_4 containing several drops of methyl red. Titrate the excess acid with ca 0.1 *N* NaOH using more indicator if necessary. Standardize the 0.1 *N* NaOH against the standard 0.1 *N* H_2SO_4 using methyl red as indicator.

[1] A Tirrill burner was converted into a semi-micro burner by inserting into the barrel of the burner a glass tube having the following dimensions: length—13.5 cm, outer diameter—9 mm, and inner diameter—7 mm.

Correct for a blank by refluxing 10 g of crystalline $MgCl_2.6H_2O$, and 1 ml of concentrated HCl, and proceeding as described above.

1 ml of 0.1 *N* H_2SO_4 = 3.003 mg of urea

The following soln containing urea and a number of other materials likely to be found in deodorants was submitted for collaborative study:

Zinc chloride, C.P.	20.0 gm
Aluminum sulfate, C.P.	20.0 gm
Magnesium oxide, C.P.	20.0 gm
Calcium carbonate, C.P.	20.0 gm
Glycerine, U.S.P.	20.0 gm
Zinc phenolsulfonate	20.0 gm
Borax	4.0 gm
Urea, C.P.	10.0000 gm
HCl and water, q.s.	2 liters

The results are presented in Tables 1 and 2.

TABLE 1.—*Analysis by Associate Referee*

WEIGHT OF UREA IN SAMPLE	RECOVERIES	
mg	*mg*	*per cent*
25.0	24.4	97.6
25.0	24.3	97.2
25.0	24.4	97.6
50.0	49.0	98.0
50.0	49.1	98.2
50.0	48.8	97.6
		Average 97.7

Results were submitted by the following collaborators:

C. Graichen, Division of Cosmetics, Food & Drug Administration, Washington, D. C.

S. Kraus and N. Shane, Bristol-Myers Company, Hillside, New Jersey.

C. F. Bruening, Food & Drug Administration, Chicago, Illinois.

S. M. Walden, Food & Drug Administration, Baltimore, Maryland.

L. S. Harrow, Division of Cosmetics, Food & Drug Administration, Washington, D. C.

The results of the collaborators vary from 92.4% to 98.2% recovery. The average of the results obtained by the five analysts was 95.4% and the largest deviations from this value were +2.8% and −3.0%.

The collaborative work reported here was confined to the analysis of a solution. However, if the product is a cream or paste, the procedure can still be applied if the sample is prepared as described in Methods of Analysis, A.O.A.C., Sixth Edition (1945), p. 46.

TABLE 2.—*Analysis by Collaborators*

COLLABORATOR NO.	WEIGHT OF UREA IN SAMPLE	RECOVERIES	
	mg	*mg*	*per cent*
1	50.0	48.5	97.0
	50.0	48.0	96.0
2	50.0	47.9	95.8
	50.0	48.0	96.0
	50.0	47.7	95.4
	50.0	48.0	96.0
3	50.0	49.1	98.2
	50.0	49.1	98.2
4	50.0	46.2	92.4
	50.0	47.0	94.0
5	50.0	46.4	92.8
	50.0	46.7	93.4
			Av. 95.4

RECOMMENDATIONS*

The Associate Referee recommends—

(1) That the proposed method for urea in deodorants be adopted, first action.

(2) That the study of deodorants and anti-perspirants be continued.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed., (1945), p. 763.
(2) MARSHALL, E. K., *J. Biol. Chem.*, **14**, 283 (1913); **15**, 487 (1914).
(3) BENEDICT, S. R., *Ibid.*, **8**, 419 (1910).
(4) FOLIN, O., *Z. physiol. Chem.*, **32**, 504 (1901).

DETERMINATION OF CHLORIDES AND SULFATES IN DEODORANTS

The anions usually found in deodorants are the chlorides and sulfates of zinc and aluminum. The chlorides can be determined gravimetrically as silver chloride; the sulfates, as barium sulfate.

* For report of Subcommittee B and action of the Association see *This Journal*, **34**, 46 (1951)

METHOD—CHLORIDES

REAGENTS

Ammonium hydroxide (1+1).
Nitric acid (1+1).
Nitric acid, 0.01 *M.*
$AgNO_3$, 0.1 *M.*

DETERMINATION

Pipet an aliquot containing about 100 mg of Cl into a 250 ml beaker. Make volume 150 ml with water, neutralize to litmus with (1+1) NH_4OH, and acidify with 1 ml of (1+1) HNO_3. If any undissolved precipitate remains add additional (1+1) HNO_3 until a clear soln is obtained. Add dropwise and with constant stirring a slight excess of 0.1 *M* $AgNO_3$. (This excess should not exceed 5 ml.) The precipitation and succeeding operations must be carried out in subdued daylight. Heat the mixture to 90–95°C. and stir until the precipitate coagulates. Allow the precipitate to settle and add 1–2 drops of 0.1 *M* $AgNO_3$ to the supernatant soln to make sure that an excess of precipitant is present. If there is no further precipitation, set the beaker aside in the dark, and allow the mixture to stand for 1–2 hours. Then decant the supernatant liquid thru a tared Gooch crucible, wash the precipitate 2–3 times with 0.01 *M* HNO_3 by decantation, and finally transfer the precipitate to the Gooch crucible using 0.01 *M* HNO_3. Continue washing the precipitate with 0.01 *M* HNO_3 until a washing gives a negative test for $AgNO_3$ when a drop of 0.1 *N* HCl is added. Complete the washing by removing most of the nitric acid with 1–2 portions of water. Dry the crucible in the oven at 120–130°C. for 2 hours. Repeat drying until successive weighings agree to 0.2 mg.

$$\text{Weight of AgCl} \times 0.2474 = \text{Weight of Cl}$$

A solution having the following composition was submitted for collaborative study:

Zinc acetate, C.P.	10.0 gm
Aluminum acetate, C.P.	10.0 gm
Boric acid, U.S.P.	10.0 gm
Magnesium carbonate, C.P.	10.0 gm
Urea, C.P.	10.0 gm
Glycerine, U.S.P.	10.0 gm
Zinc phenolsulfonate	5.0 gm
Sodium chloride, C.P.	15.0000 gm
Nitric acid and water, q.s.	2 liters

The results are presented in Tables 1 and 2.

TABLE 1.—*Analysis by Associate Referee*

WEIGHT OF Cl TAKEN	WEIGHT OF AgCl PRECIPITATE	RECOVERY	
mg	*mg*	*mg*	*per cent*
90.81	367.2	90.85	100.0
90.81	368.1	91.08	100.3
90.81	368.2	91.09	100.3
			Av. 100.2

TABLE 2.—*Analysis by Collaborators*

COLLABORATOR	WEIGHT OF Cl TAKEN	WEIGHT OF AgCl PRECIPITATE	RECOVERY	
	mg	*mg*	*mg*	*per cent*
1	90.81	365.9	90.52	99.7
	90.81	366.5	90.67	99.8
2	90.81	365.0	90.30	99.4
	90.81	365.4	90.40	99.5
3	90.81	367.4	90.90	100.1
	90.81	367.8	91.00	100.2
4	90.81	367.0	90.80	100.0
	90.81	367.4	90.90	100.1
5	90.81	366.6	90.70	99.9
	90.81	366.6	90.70	99.9
				Av. 99.9

The average recovery was 99.9% and the largest deviations from this value were −0.5% and +0.3%.

METHOD—SULFATES

REAGENTS

Ammonium hydroxide (1+1).
Hydrochloric acid, concentrated.
$BaCl_2 \cdot 2H_2O$, 1% (w/v).

DETERMINATION

Pipet an aliquot containing about 100 mg of SO_4 into a 600-ml beaker. Make volume 350 ml with water, neutralize to litmus using (1+1) NH_4OH, and acidify with 2 ml of concentrated HCl. If any undissolved precipitate remains add additional concentrated HCl until a clear soln is obtained. Heat 50 ml of a 1% soln of $BaCl_2 \cdot 2H_2O$ almost to boiling, and add rapidly with stirring to the sulfate soln which has also been heated near the boiling point. Allow the precipitate to settle, and test the supernatant liquid for complete precipitation by adding a little barium chloride soln. If there is no further precipitation set the beaker aside for 1–2 hours on the steam bath. After the digestion, decant the supernatant liquid thru a tared Gooch crucible, wash the precipitate 4–5 times with small portions of warm water by decantation, and finally transfer the precipitate to the Gooch crucible using warm water. Continue washing the precipitate with warm water until a washing gives a negative test for chlorides. Dry the crucible in the oven at 110–120°C. for 2 hours. Repeat drying until successive weighings agree to 0.2 mg.

$$\text{Weight of } BaSO_4 \times 0.4115 = \text{Weight of } SO_4$$

The following solution was prepared for collaborative work:

Sodium sulfate, C.P.	15.0000 gm
Boric acid, U.S.P.	10.0 gm

Zinc acetate, C.P.	10.0 gm
Aluminum acetate, C.P.	10.0 gm
Magnesium carbonate, C.P.	10.0 gm
Urea, C.P.	10.0 gm
Glycerine, U.S.P.	10.0 gm
Zinc phenolsulfonate	5.0 gm
Hydrochloric acid and water, q.s.	2 liters

The results are presented in Tables 3 and 4.

TABLE 3.—*Analysis by Associate Referee*

WEIGHT OF SO_4 TAKEN	WEIGHT OF $BaSO_4$ PRECIPITATE	RECOVERY	
mg	*mg*	*mg*	*per cent*
100.21	243.2	100.08	99.9
100.21	243.9	100.36	100.1
100.21	243.4	100.16	100.0
			Av. 100.0

TABLE 4.—*Analysis by Collaborators*

COLLABORATOR	WEIGHT OF SO_4 TAKEN	WEIGHT OF $BaSO_4$ PRECIPITATE	RECOVERY	
	mg	*mg*	*mg*	*per cent*
1	100.21	242.0	99.58	99.4
	100.21	241.8	99.50	99.3
2	100.21	246.4	101.39	101.2
	100.21	246.4	101.39	101.2
3	100.21	247.4	101.8	101.6
	100.21	249.8	102.8	102.6
4	100.21	246.9	101.6	101.4
	100.21	248.6	102.3	102.1
5	100.21	247.1	101.7	101.5
	100.21	246.9	101.6	101.4
				Av. 101.2

The average recovery for the five collaborators was 101.2% and the largest deviations from this value were +1.4% and −1.9%.

The collaborative work on both chlorides and sulfates was done by:

G. L. Reed, Food and Drug Administration, Baltimore, Maryland.
J. O. Millham, Division of Cosmetics, Food & Drug Admin., Washington, D. C.
C. Graichen, Division of Cosmetics, Food & Drug Admin., Washington, D. C.

S. H. Newburger, Division of Cosmetics, Food & Drug Admin., Washington, D. C.
C. F. Bruening, Food and Drug Administration, Chicago, Illinois.

The collaborative work reported here was confined to the analysis of a solution. However, if the product is a cream or paste, the procedure can still be applied if the sample is prepared as described in *Methods of Analysis*, Sixth Ed. (1945), p. 46.

RECOMMENDATIONS*

The Associate Referee recommends—

(1) That the proposed method for chlorides in deodorants be adopted first action.

(2) That the proposed method for sulfates in deodorants be adopted first action.

(3) That the study of deodorants and anti-perspirants be continued.

REPORT ON ALCOHOLIC BEVERAGES

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

This report will consist of the recommendations of the Associate Referees and the Referee as follows:†

MALT BEVERAGES, BREWING MATERIALS, AND ALLIED PRODUCTS

It is recommended—

(1) That the study of methods for determination of essential oil and resins in hops be continued.

(2) That the methods described in the report of the Associate Referee on color in beer and wort for 1950, for the spectrophotometric and photometric determination of color in beer, be adopted, first action.

(3) That a beer turbidity method commensurate and compatible with the standard reference color method (SRC) be studied.

(4) That methods either of removing or compensating for turbidity in samples for color measurement and for color in samples for turbidity measurement be studied.

(5) That methods for the degassing of beer in such a manner as to result in no change in either color or turbidity be studied.

(6) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be made official for beer. (7th Ed., *Methods of Analysis*, **10.36.**)

(7) That the method for carbon dioxide in beer, *This Journal*, **33**, 323 (1950), be made official. (7th Ed. *Methods of Analysis*, **10.23–10.27.**)

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* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 46 (1951).
† For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 52 (1951).

(8) That work be continued on polarographic and spectrographic methods for tin in beer.

(9) That studies be continued on the determination of iron in beer, giving attention to both the wet-ashing orthophenanthroline procedure and the direct non-ashing procedure as suggested by the Associate Referee.

WINES

It is recommended—

(1) That chromatographic studies of wine be continued.

DISTILLED LIQUORS

It is recommended—

(1) That the study of colorimetric methods for fusel oil be continued.

(2) That the official method, **9.29** for methanol by the immersion refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal*, **28**, 800 (1945).

(3) That study be continued on the methods for methanol in distilled liquors and drugs with a view to their correlation.

(4) That the methods for esters, aldehydes, and fusel oil be made official.

(5) That the Fulton test for caramel, *This Journal*, **31**, 77 (1948), be made official for distilled liquors and for cordials and liqueurs. (7th Ed. *Methods of Analysis*, **9.35, 9.36.**)

CORDIALS AND LIQUEURS

It is recommended—

(1) That the following methods be made official, *This Journal*, **33**, 333 (1950):

Total solids by evaporation

Caramel (Mathers test)

Citric acid (as described in Associate Referee's 1950 Report on Fruits and Fruit Products).

(2) That the rapid method for alcohol in wines and liqueurs, presented to the Association at its October, 1950, meeting, by A. D. Etienne, be studied collaboratively.

REPORT ON CHROMATOGRAPHIC STUDIES OF WINES AND DISTILLED SPIRITS

By ALEX P. MATHERS (Alcohol Tax Unit Laboratory Washington, D. C.), *Associate Referee*

New techniques in chromatography are coming into use. Chromatographic separations on sheets of filter paper permit two dimensional development. Multiple development to give maximum band separations and quantitative paper chromatography have been described by Dimmler (1). Addition of fluorescent materials to the adsorbent, such as cadmium-zinc-sulfides, is found valuable in many cases. For colorless materials indicators are employed to detect band separation.

The language of chromatography is becoming enriched by the coining of new words and terms.

Chromatopile—a column prepared by suitably clamping together a large number of filter papers placed one on top of the other.

Chromatopak—a column prepared by clamping together sheets or strips of filter paper placed side by side.

Chromatostrip (2)—a glass slide coated with a film of adsorbent material, such as 20% Plaster of Paris, 80% silica gel, 2% starch, 98% magnesium oxide, etc.

Chromatobar (2)—a casting of the adsorbent; such as the above Plaster of Paris-silica gel into a bar.

Rf Value—the ratio, distance moved by solute to distance moved by developer.

APPLICATIONS OF CHROMATOGRAPHY TO WINES

Fruit Acids:

Much work has been done in separation of organic acids by the chromatographic method (3, 4, 5, 6). It is applicable to analytical separation and identification of fruit acids in wines.

Example: The acids are absorbed on an anion-exchange column, eluted therefrom with 2% sodium hydroxide and the sodium ions removed by passage through a cation-exchange column. The eluate is concentrated by evaporation and a small amount of this acid solution is transferred to a spot on a strip of filter paper by means of a platinum loop or micropipette. Successive applications are applied to the same spot, drying between applications until a sufficient quantity of the acids has been adsorbed on the paper. The paper is suspended in a graduate with lower tip immersed in developing solution. After development and drying, the paper is sprayed with brom-cresol green indicator to detect the acid spots.

Ammonium vanadate solution is somewhat specific when used as an indicator for fruit acids. Tartaric turns red, citric, malic, and some others turn yellow, while oxalic acid soon turns blue.

For identification work, the Rf values are valuable. A known acid may be chromatographed on the same paper strip simultaneously with the unknown to determine if it is one of the acid components in the sam-

ple. If absolute Rf values are to be used for identification, it is necessary to rigidly control the system. It may be practical to formulate a chromatographic system so that organic acids will have definite Rf values which will serve for identification purposes.

The acids may be readily eluted from the paper strip but to obtain quantities large enough for other identification techniques it is usually necessary to resort to column chromatography.

Anthocyanins:

Most of the antocyanins in wine are broken down into anthocyanidin and sugar fractions during fermentation and preliminary treatment of the sample. It is desirable to remove most of the sugar, pectin and other solid material in the wine before attempting chromatographic separations.

One method of preliminary purification of the anthocyanidins consists in extraction of the colored pigments from a salt solution of the wine by phenol, pyridine, isopropyl or higher alcohols. The extract may be evaporated to concentrate the colors or they may be adsorbed on a column of silica gel after addition of benzene. When a column is used it may be possible to separate the pigments by use of some of the developers listed below. Total elution can be obtained by formic acid-methanol (2:98).

Example of extraction procedure: To 25 ml of wine add 10 g sodium bisulphate and extract with two 15 ml portions of benzyl alcohol.

The following purification procedure is probably more generally applicable. To 20 ml of wine add 20 ml of calcium chloride-methanol solution (1 g/100 ml). Pipette 1 ml of concentrated ammonia into solution and centrifugalize. The supernatant liquid contains at least a portion of the yellow flavones but no study has been made here on this fraction. The precipitate is treated with 20 ml of phosphoric acid-isopropyl alcohol solution (1 ml 85% H_3PO_4/100 ml) followed by centrifugation to remove the calcium phosphate. To the supernatant liquid is added two volumes of ethyl ether. The solution is passed through a column of silicic acid and further developed with phosphoric acid-isopropyl alcohol-ethyl ether solution (1:99:200). A similar anthocyanidin separation has been reported by Spaeth and Rosenblatt (7). Fractional elution may be carried out by decreasing the ethyl ether content of the developer. To complete elution it may be necessary to use formic acid-methanol (2:98) or perhaps water.

A number of tests for identifying the anthocyanidins are listed in Gilman (8). Other useful indicators are: solutions of aluminum, ferric, zinc and chromium chlorides, bromine and ammonia vapors.

DEVELOPERS

Neutral Type: Various combinations of neutral solvents, such as water, alcohols, esters, ethers, etc.

Example: Fusel Oil-Ethyl Acetate, 2:1 (two parts first: one part second). Ethyl Alcohol-Ethyl Acetate-Water, 2:10:5.

Acidic Type: Various combinations of neutral solvents with acids: such as formic, acetic, p-toluene sulfonic, 0.1 *N* hydrochloric.

Example: Phenol-Water, 1:1. t-Amyl Alcohol-Water-p-Toluene sulfonic acid, 6:3:0.2.

Basic Type: Various combinations of neutral solvents with bases; such as ammonia, aminies, collidine, etc.

Example: Collidine-Water, 1:1. Pyridine-Concentrated Ammonia-Water, 6:2:1.

APPLICATIONS OF CHROMATOGRAPHY TO DISTILLED SPIRITS

Only preliminary work has been done in this laboratory on chromatography of whisky colors. An ethyl ether extract of whisky diluted with an equal volume of petroleum ether was passed through a column of silicic acid and the chromatogram developed with ethyl ether followed by ethyl ether-ethyl alcohol. Four color components have been obtained from the ether extract.

RECOMMENDATIONS*

A study of the material published in this field to date does not seem to warrant recommendation of any specific chromatographic techniques for routine analysis of wines and distilled spirits. With a view to establishing suitable routine procedures in the field of chromatography, it is recommended that collaborative investigations be conducted.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 52 (1951).

REPORT ON METHANOL

COLORIMETRIC DETERMINATION OF METHANOL IN SOLUTIONS CONTAINING ETHYL ALCOHOL

By JAMES F. GUYMON

(University of California, Davis, California), *Associate Referee*

Many methods have been proposed for the determination of methanol. Gettler (1) has listed 58 different tests for methanol in the literature, 46 of which were based upon reactions of various substances with formaldehyde (formed from methanol by oxidation). He studied the reliability of these methods as applied to 18 different alcoholic liquors and recommended 7 tests, including the reduced fuchsin color reaction. All tests recommended were based upon reactions of formaldehyde. Gettler also tried 11 methods for oxidation of methanol to formaldehyde and found the dichromate and permanganate methods preferable.

Most of the procedures described in the recent literature, especially those applied to methanol in the presence of ethyl alcohol, are based upon the method of Denigès (2). His procedure contains three essential steps: A. oxidation of methanol with potassium permanganate to formaldehyde; B. removal of the excess permanganate with oxalic acid; and C. development of a violet coloration by the interaction of formaldehyde with a solution of fuchsin reduced with sulfite (Schiff's reagent) in the presence of sufficient sulfuric acid to suppress any coloration from acetaldehyde. Numerous modifications of this method have resulted from attempts to improve upon one or more of the following factors: A. stability of Schiff's reagent; B. concentration of ethyl alcohol; C. efficiency of oxidation of methanol to formaldehyde; D. increase of sensitivity for methanol or elimination of interference from other substances; and E. influence of temperature upon color development.

The two present official methods, **16.25–16.28,** (3) for methanol in distilled liquors, and **39.161–39.162,** (4) for methanol in drugs, are both derived from the Denigès method. The principal publications leading to the two present procedures are reviewed in order that their divergencies may be reconciled or understood. The General Referee for alcoholic beverages has recommended (5) that both procedures be investigated with a view to bringing about conformity in the procedures, so far as possible.

The original Denigès procedure (2) specified the following steps: A. to 1 ml of the alcohol to be examined, add 5 ml of 1 per cent $KMnO_4$ solution, 0.2 ml of pure H_2SO_4, and mix; B. after 2 or 3 minutes add 1 ml of oxalic acid solution (about 8 per cent) shake and allow to clear; and C. after adding 5 ml of fuchsin bisulfite solution, mix and allow to stand. Denigès pointed out that ethyl alcohol increased the efficiency of the test,

so for aqueous solutions of methanol alone, he recommended adding 0.1 ml of 95 per cent ethyl alcohol.

Simmonds' modification (6) called for 5 ml of the test solution to be oxidized with 2.5 ml of 2 per cent $KMnO_4$ solution with the same quantity of acid and time of oxidation as that of Denigès, but with only 0.5 ml of oxalic acid solution (9.5 per cent). Then to the cleared solution Simmonds added 1 ml of strong sulfuric acid prior to the addition of 5 ml of Schiff's reagent. For convenience, he chose 10 per cent as the alcoholic strength of the mixture for oxidation.

Elvolve (7) demonstrated that this test for methanol was more sensitive when the ethyl alcohol was reduced from 10 per cent to 0.5 per cent by volume. Elvolve also initiated the use of anhydrous sodium sulfite in preparation of Schiff's reagent, and his instructions for preparation of this reagent are essentially those of the present official methods. The instructions for the complete procedure given by Elvolve are otherwise almost identical to those of Simmonds.

The next study and modification of the Denigès test in the sequence of papers directly leading to the official methods was that of Chapin (8). He decided that only 0.04 ml of total alcohols, made to a volume of 5 ml prior to oxidation, should be standard (equivalent to 5 ml of 0.80 per cent alcohol). Chapin studied the oxidation of methanol and ethyl alcohol and concluded that acid dichromate solutions result in a high yield of acetaldehyde from ethyl alcohol but a low yield of formaldehyde from methanol in comparison with acid permanganate solutions. With permanganate, the nature and proportion of the acid used was found important, the highest yield of formaldehyde resulting generally from slow oxidation in the presence of low hydrogen ion concentration. Phosphoric acid was found preferable to sulfuric acid. Chapin's use of phosphoric acid has been retained by most subsequent authors. In order to obtain maximum yield of formaldehyde, he found also that 3 per cent was the appropriate strength of potassium permanganate when the volume used was 2 ml. For his quantitative method the following procedure was recommended: to 4 ml of the alcohol solution add 1 ml of H_3PO_4 solution (85% diluted 1 to 5) and 2 ml of 3 per cent $KMnO_4$, and oxidize 30 minutes; add 1 ml of 10 per cent oxalic acid, followed by 1 ml of concentrated H_2SO_4, and after allowing to stand a few minutes for cooling, add 5 ml of Schiff-Elvolve reagent and develop the color for 0.5 to 2 hours. Chapin also described a qualitative test for methanol in which the alcohol content was 5 per cent by volume in the test solution.

The procedure outlined by Chapin for qualitative work was employed, essentially, by Georgia and Morales (9), primarily for detection of methanol in alcoholic beverages and the effect of interfering substances rather than for quantitative measurement. Consequently, they employed

a procedure utilizing 5 ml of the alcohol solution of 5 per cent by volume total alcohols. This point is emphasized, since the quantity of total alcohol present is a major point of difference between the A.O.A.C. methods **16.28** (3) and **39.162** (4).

Georgia and Morales used the Elvolve procedure for preparing Schiff's reagent except that they substituted rosaniline hydrochloride for fuchsin. They also simplified the procedure by combining certain of the reagents. Beyer (10) used the Georgia and Morales procedures, but he found by experiment that the color produced was maximum at about 22 per cent total alcohols, expressed as concentration in a 0.25 ml sample. This was diluted with water to 5 ml prior to addition of oxidizing solutions; actually then, 1.1 per cent total alcohols was contained in the test solution. A.O.A.C. procedure **16.25–16.28** (3) is based upon this paper of Beyer. He employed a 15 mm diameter, 19 inch length column, packed with single turn glass helices to concentrate the methanol in ethyl alcohol solutions into approximately one-third the original volume.

The A.O.A.C. method **39.161, 39.162** (4) for methanol (0.5 to 5.0 per cent) in ethyl alcohol for drugs is based upon the report of Bailey (11). Aside from steps in preparation of the sample, this method differs from the method for distilled liquors in the following regards: A. fuchsin is specified instead of rosaniline hydrochloride; B. 5 ml of 5 per cent total alcohols is present in the sample instead of 5 ml of 1.1 per cent; C. the concentrations of phosphoric and sulfuric acids differ; and D. the method of measuring the color developed is different.

The question of the reliability of the method for methanol was raised by Morison (12). Possible reasons for his inconsistent results were pointed out in a previous report (13). Morison used standard solutions prepared in accordance with A.O.A.C. procedure **39.161**, but used the test procedure of Georgia and Morales, which is essentially that of A.O.A.C. method **16.28** as modified by Beyer.

In the earlier publications color comparisons were normally made visually with a series of standards prepared at the same time. Nowadays analysts are inclined to employ photoelectric colorimeters or photometers. Such instruments facilitate analysis of many more samples and make possible greater precision. However, neglect of the influence of factors such as time, temperature, instrument sensitivity, etc., may lead to erratic results when obtained from a standard graph of readings *vs.* concentration which were prepared at some other time. Consequently, it was considered advisable to study systematically first, the several physical and chemical factors affecting the method using known solutions of methanol and ethyl alcohol, and then, the efficacy of recovery and preparation of samples prior to adoption of a standard procedure for a collaborative study. The method follows.

METHANOL

PROCEDURE

Reagent solutions:

(*a*) *Potassium permanganate.*—Dissolve 3.0 g of $KMnO_4$ and 15 ml of 85 per cent H_3PO_4 in sufficient water to make 100 ml of soln.

(*b*) *Oxalic-sulfuric acid.*—Dissolve 5.0 g of $H_2C_2O_4$ in sufficient H_2SO_4 (1+1) to make 100 ml of soln.

(*c*) *Modified Schiff's reagent.*—Dissolve 0.2 g of rosaniline hydrochloride in about 5 ml of 95 per cent ethyl alcohol plus a few ml of water with warming. Dilute to about 150 ml with water, add 2.0 g of anhydrous Na_2SO_3 dissolved in 20 ml of water. Add 2.0 ml of concentrated HCl, dilute to 200 ml and store in the refrigerator for 24 hours prior to use. If the soln is not completely decolorized, add a sufficient measured quantity of activated carbon, shake, and after 10 min., filter. When the reagent is not in use store in the refrigerator and renew after 4–6 weeks. For Eastman rosaniline hydrochloride, 10 mg of Nuchar WW per 100 ml is adequate for preparation of a water-white reagent. The same quantity and type of carbon should be used in making up subsequent lots of the reagent.

Alcohol solutions:

(*a*) *Ethyl alcohol.*—Dilute 10 ml of 95 per cent by volume ethyl alcohol to 1 liter. Blanks containing 0.95 per cent ethyl alcohol developed by the above procedure using both U.S.P. spirits and the middle third fraction from U.S.P. spirits diluted and redistilled in a 0.5-inch diameter, 4 foot column packed with single turn glass helices gave negative tests for methanol.

(*b*) *Standard solutions of methanol* in 0.95 per cent ethyl alcohol. Merck reagent methanol (methyl alcohol), redistilled (middle cut), was used. Dilute 1.000 g of methanol to 1 liter with 0.95 per cent ethyl alcohol (1 ml = 1 mg of methanol). Prepare dilute standards containing 2, 4, 6, 8, and 10 mg per 100 ml in 0.95 per cent ethyl alcohol.

Apparatus.—A Beckman spectrophotometer, Model DU with 10 mm Corex cells, was used for transmittancy curve readings. A Coleman spectrophotometer, Model 11, was employed for color readings at 575 mμ with Coleman square cuvettes, 13.1 mm optical path. For deeply colored solns, the optical path was reduced to 1.9 mm, where herein indicated, with glass prism plugs. A Klett-Summerson colorimeter using Klett round tube cells and a KS-56 filter was tested and found suitable for the conditions adopted (see Figure 10).

DETERMINATION

Pipet 5 ml of the alcohol soln to be tested, containing about 0.25 mg of methanol (5 mg per 100 ml), and 0.95 per cent by volume ethyl alcohol, into a 25×200 mm test tube. Add 2 ml of the $KMnO_4$ soln, swirl to mix, and allow to oxidize for 20 min. in a 25°C. water bath. Follow with 2 ml of the oxalic-sulfuric acid soln; after the contents are completely decolorized, add 5 ml of modified Schiff's reagent, stopper, mix by inverting 3 times, and let stand in the 25°C. water bath for 2 hours. Obtain the percentage transmittancy readings with the spectrophotometer at 575 mμ or in a colorimeter using appropriate filter. Determine the content of methanol from a curve of concentration *vs.* percentage transmittancy readings obtained with known standards under exactly the same conditions.

EXPERIMENTAL RESULTS

Transmittancy Curve

Maximum absorption was found to occur at 575 mμ. The plot of Figure 1 was made from readings using the Beckman spectrophotometer (10 mm Corex cells) and a solution containing 7.92 mg of methanol per 100 ml, but with color development at 20°C. for 1.5 hours. The curve closely resembles the abridged curve of Beyer (10) obtained with a series of color filters and a neutral wedge photometer. Transmittancy curves obtained

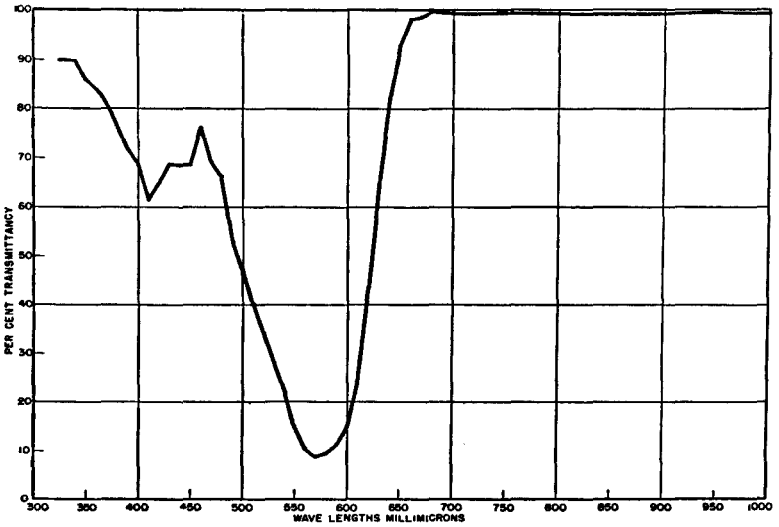


FIG. 1.—Transmittancy curve.

with other concentrations of methanol and after various times of color development, using both the Beckman and Coleman spectrophotometers, indicated that no shift occurs in the position of maximum absorbency.

Oxidation with Potassium Permanganate

The time specified for the oxidation step by various authors (2, 6, 7, 8, 9, 10, 11) varies from 2 or 3 minutes to 30 minutes. Both A.O.A.C. procedures call for 10 minutes. Four different methanol solutions were oxidized for varying periods of time and the color developed for two hours at 25°C. The absorbency (optical density or extinction) values are shown in Figure 2. The solutions in which ethyl alcohol was present developed more color with increased time of oxidation up to 30 minutes. However, 20 minutes was considered satisfactory and adopted as standard. The data show that the time of the oxidation period should be accurately meas-

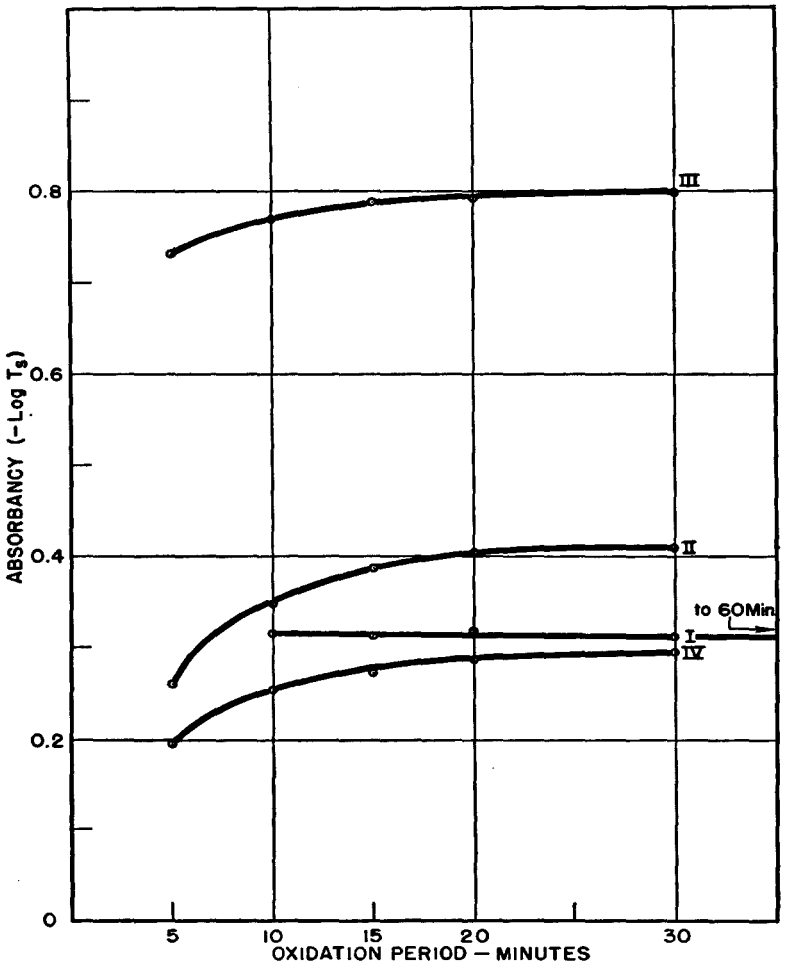


FIG. 2.—Effect of time of oxidation.

I—0.02% CH_3OH, 0.0% C_2H_5OH, 13.1 mm optical path
II—0.02% CH_3OH, 0.95% C_2H_5OH, 1.9 mm optical path
III—0.02% CH_3OH, 4.75% C_2H_5OH, 13.1 mm optical path
IV—0.004% CH_3OH, 0.95% C_2H_5OH, 13.1 mm optical path.

ured. The solution containing only methanol showed no appreciable difference in a period of from 10 minutes to one hour.

The amount of potassium permanganate required was studied. Table 1 includes the data obtained with five methyl-ethyl alcohol mixtures which

TABLE 1.—*Influence of volume of potassium permanganate solution[1] upon absorbency*

METHANOL (VOL. %)	ETHYL ALCOHOL (VOL. %)	ABSORBENCY (−Log T)				
		ML OF 3% $KMnO_4$ REAGENT				
		1	2	3	4	5
0.004	0.95	0.047	*0.144*	0.108	0.043	0.019[2]
0.02	0.0	*0.242*	0.156	0.065[2]	0.029[3]	0.026[4]
0.02	1.43	0.616	1.155	*1.252*	1.065	0.616[2]
0.02	4.75	0.162	0.419	0.708	0.955	*0.959*[2]
*0.10	1.90	0.597	1.009	*1.155*	*1.155*	1.071

* 1.9 mm optical path.
[1] Final volume of solution prior to addition of Schiff's reagent was 15 ml, 6 ml more than standard procedure.
[2] Required 3 ml of oxalic-sulfuric acid solution.
[3] Required 4 ml of oxalic-sulfuric acid solution.
[4] Required 5 ml of oxalic-sulfuric acid solution.

were oxidized with varying volumes of the standard 3 per cent $KMnO_4$-H_3PO_4 reagent. Total volumes were equalized with appropriate amounts of water at the beginning of oxidation. Maximum absorbency readings resulting are italicized in the table. It is evident that increasing concentrations of ethyl alcohol required higher amounts of permanganate in order to produce maximum coloration. For the solution containing 0.95 per cent ethyl alcohol the standard volume of 2 ml of permanganate reagent was optimum.

Consideration was given to the nature of the oxidation with permanganate as well as to its sufficiency for different concentrations of alcohol mixtures. It is apparent that manganese dioxide is precipitated in the tube during oxidation. When the contents of the tube after the oxidation period are rinsed into a flask and 5 ml of 20 per cent potassium iodide added, the manganese is completely reduced and the iodine released may be titrated against sodium thiosulfate. Table 2 contains the titration values for a series of tubes containing 0.02 per cent by volume methanol plus ethyl al-

TABLE 2.—*Residual oxidizing capacity toward iodide after 10-minute oxidation of alcohol mixtures*

ETHYL ALCOHOL (% BY VOLUME)	$Na_2S_2O_3$ (ML .05 N)
0.0	36.6
0.48	28.8
0.95	16.3
1.42	12.6
1.90	11.9
2.85	11.0
4.75	10.7
7.12	10.6
9.50	10.5

cohol as indicated, which were oxidized for 10 minutes at 25°C. using 2.0 ml of the 3 per cent potassium permanganate reagent. With increasing alcohol concentration the residual oxidizing capacity decreased to 10.5 ml of 0.05 *N*. If managanese dioxide was entirely the end product of the reduction of permanganate, the titer of a completely reduced tube measured against iodide would be $\frac{2}{5}$ of 36.6 or 14.6 ml of .05 *N*. Therefore, part of the potassium permanganate is reduced to manganous ion (Mn^{++}) by the alcohols.

In 2.0 ml of the oxidizing solution 0.060 g of potassium permanganate are present. The theoretical amounts necessary for completely oxidizing the ethyl alcohol in one tube to acetaldehyde are:

ETHYL ALCOHOL	$KMnO_4$, g	
(VOL. %)	REDUCED TO $-Mn^{++}$	$-Mn^{++++}$
0.48	0.026	0.044
0.95	.052	.087
1.90	.104	.174
4.75	.261	.435
9.50	0.522	0.870

Thus the oxidant is theoretically adequate in quantity for no more than about 1 per cent total alcohols. However, even when permanganate is insufficient, as for 5 per cent total alcohols, it is not preferentially consumed entirely by either methyl or ethyl alcohols. Instead, some of both are oxidized so that the color test for formaldehyde constitutes an empirical basis for the methanol determination provided the ethyl alcohol content is kept the same.

Since the oxidizing reagent also contains acid, the results of Table 1 were likely influenced, at least quantitatively, by this factor as well as by the necessary volume increase (6 ml more than the regular procedure). Therefore, three oxidizing solutions were prepared containing 15 ml of 85 per cent phosphoric acid each and 2, 3, and 4 grams of potassium permanganate per 100 ml, respectively. These reagents were employed to oxidize alcohol solutions containing a fixed amount of methanol (0.02 per cent by volume or 15.8 mg per 100 ml) and variant quantities of ethyl alcohol. Two ml of oxalic acid solutions containing 3, 5, and 6.7 grams per 100 ml in sulfuric acid (1+1) were used to decolorize, respectively, the tubes in which 2, 3, and 4 per cent $KMnO_4$ reagents were added. Thereby the final volumes were equal and the same as in the standard method. The color was developed for 2 hours at 25°C. Results are shown in Figure 3. It is clear that increasing ethyl alcohol contents require larger quantities of oxidant for optimum color development. For 5 per cent total alcohols, the sensitivity is reduced even with 4 per cent $KMnO_4$. But with 0.95 per

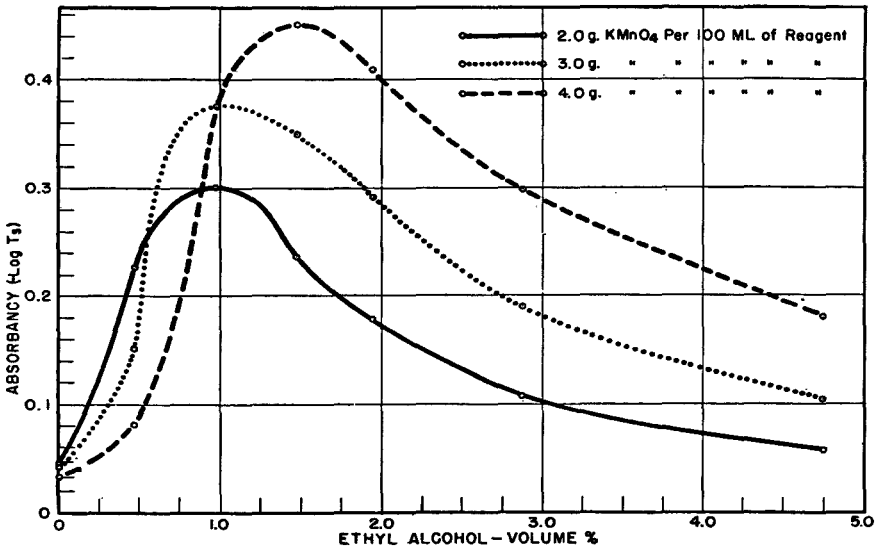


FIG. 3.—Effect of concentration of potassium permanganate in the oxidizing solution.

cent alcohol, 3 per cent $KMnO_4$ is adequate for the conditions of this procedure. Quantities of oxidant larger than those employed would necessitate increases in volumes of both the oxidizing and the reducing reagent solutions, which it was desired to avoid.

Effect of Acid Concentration

The procedure calls for 2 ml of oxalic-sulfuric acid reagent which is 18 *N* with respect to sulfuric acid. In one experiment, tubes containing 0.02 per cent methanol and 1.4 per cent ethyl alcohol by volume were treated in the standard manner except that 2 ml of 5 per cent oxalic acid in water plus the volumes of 18 *N* sulfuric acid indicated in Table 3 were used. Total volumes were made equal with water (total volume was 6 ml greater than in the standard procedure). The quantity of 18 *N* sulfuric acid contained in the standard oxalic-sulfuric acid reagent (2 ml) gives more color than either more or less acid.

TABLE 3.—*Effect of sulfuric acid concentration upon color intensity*

H_2SO_4 (ML OF 18 *N*)	ABSORBENCY (−Log Ts)
1	1.041
2 (standard)	1.119
4	0.848
6	0.597

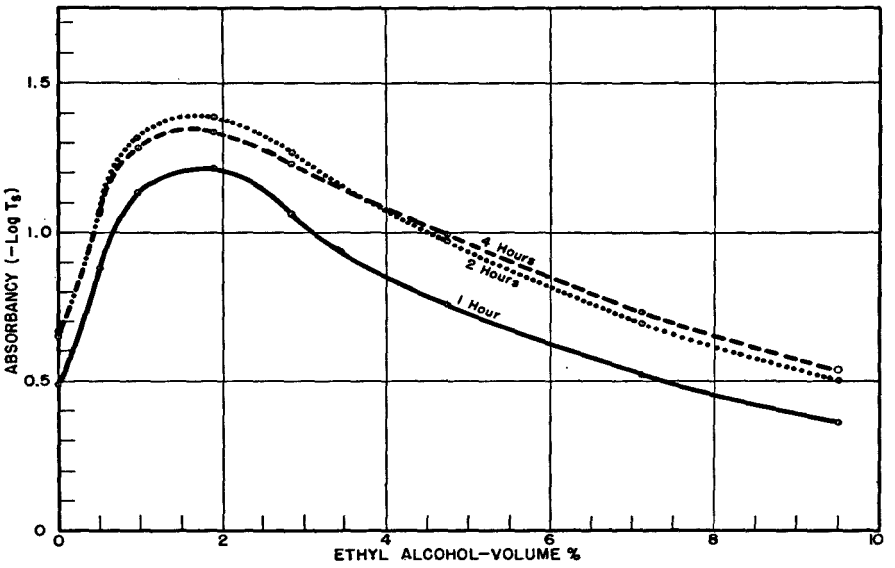


FIG. 4.—Effect of concentration of ethyl alcohol upon color after 1, 2, and 4 hours.

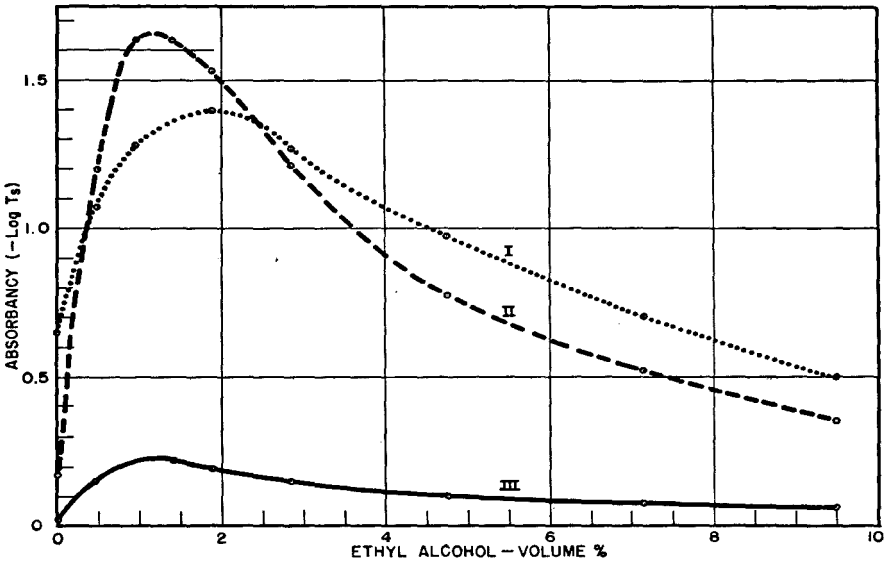


FIG. 5.—Effect of ethyl alcohol at three levels of methanol content.

I—0.1% CH_3OH, 1.9 mm optical path
II—0.02% CH_3OH, 13.1 mm optical path
III—0.004% CH_3OH, 13.1 mm optical path.

Effect of Ethyl Alcohol Concentration

The influence of the amount of ethyl alcohol has already been mentioned in connection with the study of the oxidation step. The factor was further studied in relation to time of color development and with different quantities of methanol present.

Figure 4 shows the results found for a series of alcohol mixtures each containing 0.02 per cent by volume (15.8 mg/100 ml) of methanol oxidized for 10 minutes, with the readings made at 1, 2, and 4 hours of color development at 25°C. The curves of Figure 5 show similarly the absorbency values for three series of mixtures, each of different methanol con-

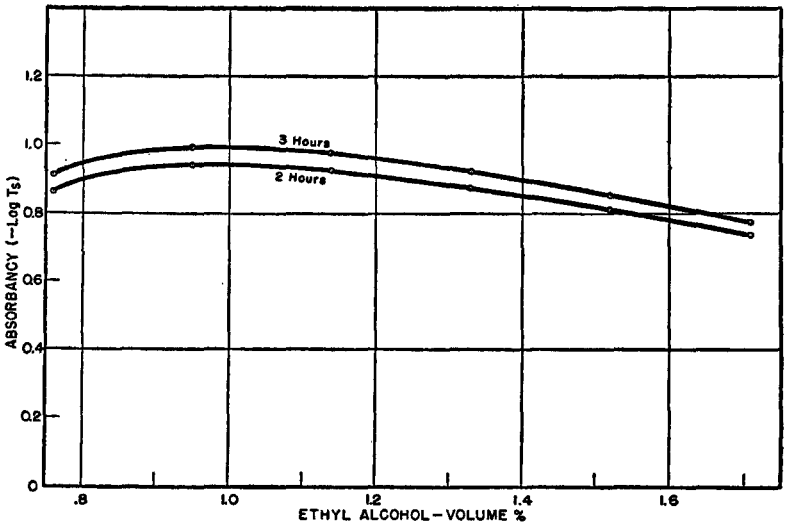


FIG. 6.—Effect of dilute concentrations of ethyl alcohol.

centrations read at 2 hours. In all cases maximum color intensity occurred at about 1 to 2 per cent ethyl alcohol. For higher methanol concentrations there was some indication of a shift of the maximum color toward a slightly higher ethyl alcohol concentration.

Finally, in order to fix more exactly the ethyl alcohol content for greatest sensitivity, another series of solutions of ethyl alcohol, differing by 0.2 per cent and each containing 0.01 per cent by volume of methanol, were prepared and the color developed according to the regular procedure. The curves of Figure 6 are the result in which the maximum occurred at 0.95 per cent ethyl alcohol.

Effect of Time and Temperature upon Color

Aliquots of two solutions of methanol, 0.004 and 0.02 per cent by volume in 0.95 per cent ethyl alcohol, were oxidized for 20 minutes at 25°C.

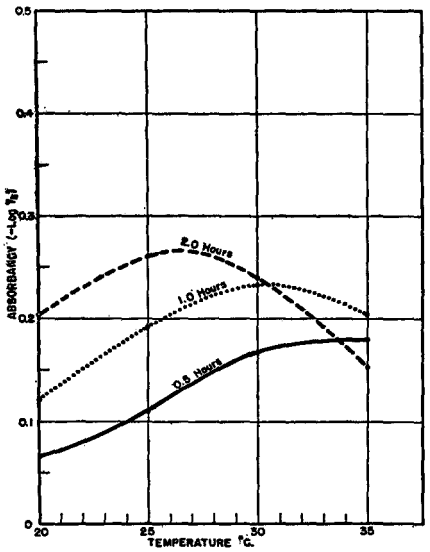


FIG. 7.—Effect of temperature upon color development for 0.004 per cent methanol (b = 13.1 mm).

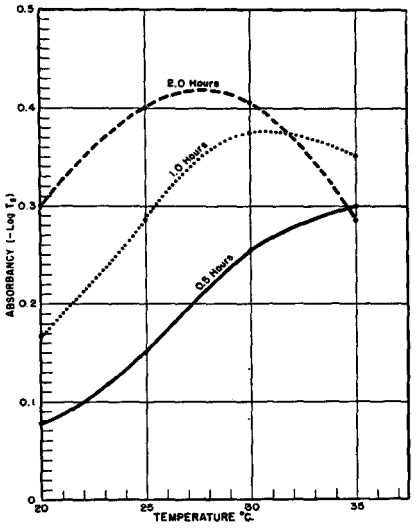


FIG. 8.—Effect of temperature upon color development for 0.02 per cent methanol (b = 1.9 mm).

but with color development at different temperatures as shown in Figures 7 and 8. Absorbency readings were made at 0.5, 1.0, and 2.0 hours. It is noted that the color develops more rapidly at higher temperatures but also begins to deteriorate earlier. Furthermore, the greatest absorbency occurred at the lower temperature of 25°C. with two hours' development; hence this combination was adopted as standard. If the time of standing were to be reduced to one hour, then 30°C. would be indicated, with only a slight decrease in sensitivity.

DISCUSSION

These studies clearly establish the interdependence of several factors affecting the sensitivity and reproducibility of this colorimetric determination for methanol. Controlled conditions are particularly important when photometric methods are employed and transmittancy or absorbency readings are translated to percentage of methanol from a previously prepared standard curve. Even for visual or instrumental comparison of unknowns with a set of standard tubes prepared simultaneously, control of conditions such as time and temperature of oxidation and color development may not be disregarded.

The objects of these studies were to establish conditions for greatest sensitivity and to demonstrate the magnitude of influence from condition variables. For some other objective another set of conditions might serve equally well or better. As an example, prime consideration might be given

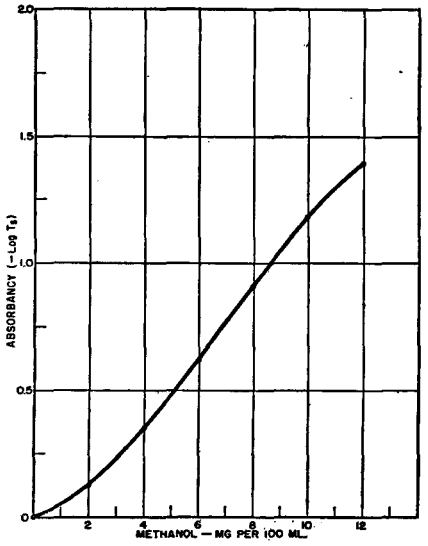


FIG. 9.—Standard curve of methanol concentration for Coleman Model 11 spectrophotometer (b = 13.1 mm).

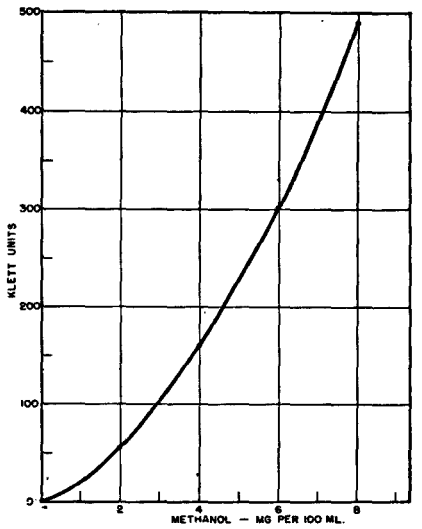


FIG. 10.—Standard curve of methanol concentration for Klett-Summerson photoelectric colorimeter, round tube cells.

to finding a straight line relationship between concentration and absorbency. Readings at 575 mμ with the spectrophotometer or in the Klett-Summerson instrument with the KS-56 filter and Klett round tube cells do not give straight lines. Standard curves for both instruments are shown in Figures 9 and 10 to illustrate their general character. Analysts, of course, should prepare standard curves for their own instruments.

Schiff's Reagent

Beyer (14) called attention to the fact that currently available rosaniline hydrochloride or basic fuchsin could not be completely decolorized by sodium sulfite and hydrochloric acid in preparation of Schiff's reagent. Instead, a brownish or amber color remained, the removal of which was accomplished by shaking for 10 minutes with 0.5 gram of Darco activated carbon added to 250 ml of the reagent, followed by filtering. This is confirmed by the writer. However, by using Eastman rosaniline hydrochloride considerably less carbon is sufficient to secure water-white reagents. About 10 mg of Nuchar WW carbon per 100 ml of reagent removed the amber color; this amount was subsequently used for preparation of the reagent.

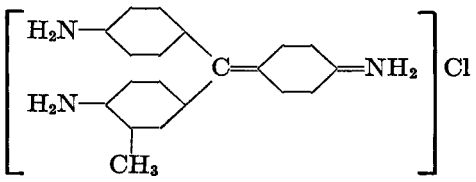
Furthermore, a considerable excess of carbon reduces the color developing capacity, as seen in the second column of Table 4. In fact, a large unmeasured excess of carbon in a preliminary experiment completely removed the color-forming capacity of the reagent.

TABLE 4.—*Effect of activated carbon upon color developing capacity of Schiff's reagent*

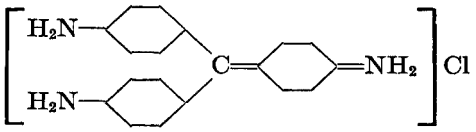
AMOUNT OF NUCHAR WW (MG/100 ML OF SCHIFF'S REAGENT)	ABSORBENCY (−Log Ts)	
	0.02% CH_3OH (b=1.9 MM)	BLANK (b=13.1 MM)
0	0.389	1.990
10	0.396	2.000 (reference)
25	0.387	2.001
50	0.367	2.004
200	0.340	2.000

The question arises of the difference, if any, between basic fuchsin and rosaniline hydrochloride for preparation of Schiff's reagent, since the former has been specified by some authors (2, 4, 6, 7, 8) and the latter by others (9, 10). Basic fuchsin is defined as a mixture of rosaniline and pararosaniline hydrochlorides by Hackh's Chemical Dictionary, third edition, by the Merck Index, fifth edition, and by the U. S. Pharmacopoeia, thirteenth revision. However, the Eastman Organic Chemicals List No. 37 (1950) indicates the same chemical formula for rosaniline hydrochloride as for basic fuchsin. The latter is listed as a certified stain and is somewhat more expensive. Some of the authors referred to herein considered both dyes as synonymous or at least equivalent for the purposes of preparing the reagent. Also, some texts of organic chemistry (Karrer, Whitmore) identify fuchsin as rosaniline hydrochloride. Rosaniline differs from pararosaniline in that it contains a methyl group substituted in one of the phenyl rings:

Rosaniline



Pararosaniline



Schiff's reagents were prepared from Eastman and National Aniline rosaniline hydrochlorides and Coleman and Bell basic fuchsin, using 10 and 25 mg of Nuchar WW per 100 ml for removing residual color. That made from the National Aniline was not completely decolorized, even

with 25 mg of carbon, but retained a yellow color. A solution containing 4 mg of methanol in 0.95 per cent ethyl alcohol was used to compare these reagents as to color developing capacity. The absorbency values, using respective blanks for reference, were:

	NUCHAR WW USED	ABSORBENCY
Rosaniline hydrochloride (Eastman)	10 mg	0.373
Rosaniline hydrochloride (National Aniline)	10	.272
	25	.328
Basic fuchsin (Coleman and Bell)	10	.446
	25	0.418

Basic fuchsin thus gave a somewhat more intense color. Transmittancy curves obtained with the Coleman spectrophotometer showed maximum absorption at 575 mμ for both fuchsin and rosaniline hydrochloride, although the fuchsin absorbed slightly stronger toward the red end of the spectrum (at less than 575 mμ).

For the colorimetric determination of methanol it appears that both basic fuchsin and rosaniline hydrochloride should be specified by the official methods.

Age of Reagents

Schiff's reagent was found to keep quite well when stored at 0°C. while not in use. Three reagents of different ages were compared at the same time on two standard solutions of methanol and gave the following absorbency readings:

CH_3OH PER 100 ML, MG	AGE (DAYS)		
	1	23	53
4	0.371	0.374	0.382
6	0.658	0.674	0.678

There was a slight increase in degree of absorbency with the older reagents.

Three permanganate-phosphoric acid solutions, 2 to 58 days in age and kept at laboratory temperatures, showed essentially the same capacity for oxidation of a methanol-ethyl alcohol solution as determined by the absorbency values after color development.

Efficiency of Recovery by Distillation

The official method, **16.25–16.28,** directs that a 25 ml sample containing about 50 per cent ethyl alcohol be distilled in a laboratory fraction-

ating column of 15 mm diameter and 475 to 500 mm long, packed with single-turn glass helices in a specified manner. A distillate is produced, about 8.5 ml in volume, containing approximately 94 per cent total alcohols. Presumably this would concentrate all of the methanol into a fraction containing about 64 per cent of the ethyl alcohol originally present. The resulting concentration of methanol with respect to ethyl alcohol would be approximately 1.56 times. Since the distillate is to be subsequently diluted to a definite content of total alcohols, the question arises as to how much benefit is to be derived from the relatively tedious and time-consuming fractional distillation. Furthermore, it was considered important to seek confirmation of whether or not complete separation of

TABLE 5.—*Recovery of methanol by fractional distillation*

FRACTION	ETHYL ALCOHOL (VOL. %)		METHANOL (g PER FRACTION)	
	RUN NO. 1	RUN NO. 2	RUN NO. 1	RUN NO. 2
1	94.5	96.0	0.217	0.247
2	96.0	96.0	0.109	0.098
3	95.7	95.7	0.081	0.058
4	95.9	95.8	0.057	0.046
5	94.6	93.4	0.022	0.024
6	13.5	2.4	0.000	0.002
7	0.4	0.0	0.001	0.000
8	0.0	—	0.000	0.000
	Total methanol recovered		0.487 g	0.475 g
	Per cent recovery		97.4	95.0

methanol from the ethyl alcohol, eliminated as residue and holdup, results. The following series of experiments were conducted to test the efficiency of recovery.

Into a 500-ml boiling flask was measured 250 ml of a mixture containing 0.200 g of methanol per 100 ml in 50 per cent ethyl alcohol. It was refluxed and distilled in a packed column as specified in the official method (3). Eight 25-ml fractions were collected and analyzed for methanol. The data for two separate runs are given in Table 5, average recovery being about 96 per cent.

By interpolation between appropriate fractions it was calculated that the per cent recovery of methanol was 86 per cent and 85 per cent, respectively, for runs 1 and 2 when the total volume distilled was 85 ml—the same ratio of volumes as in the A.O.A.C. procedure.

Next two separate distillations were made of 25 ml samples of the same alcohol mixture as before, collecting 8.3 to 8.5 ml of distillate. Analysis of each gave 82.0 and 82.2 per cent recovery, respectively. That the frac-

tional distillation was giving incomplete recovery is shown by the following analysis made upon aliquots of the same alcohol mixture (0.2 g CH_3OH in 50 per cent C_2H_5OH):

	RECOVERY *per cent*
(a) Analyzed directly	101.0
(b) Distil 50 ml of the alcohol mixture in a simple still collecting about 40 ml, make to 50 ml and analyze	100.0
(c) Distil 50 ml plus 10 ml of water as in (b) collecting 50 ml of distillate and analyze	102.5

These results clearly show that the fractional distillation step fails to concentrate all the methanol in the first 8.5 ml of distillate from a 25-ml sample. Because of this and the considerations already mentioned, there seems no justification for the step.

SUMMARY

The pertinent literature leading to the present two official methods for the determination of methanol have been reviewed and the differences in procedures noted. An investigation was therefore made of the influence of condition variables including the quantity of potassium permanganate used for oxidizing, length of the oxidation period, the concentration of ethyl alcohol present, and time and temperature of color development. The intensity of the color developed was found to be affected by all of these factors. The procedure described incorporates control of these variables and allows the determination of as little as 1 mg of methanol per 100 ml of 0.95 per cent alcohol solutions. Little difference in the color development was obtained with basic fuchsin and rosaniline hydrochloride.

The efficiency of methanol recovery by the distillation step described in the official method **16.25–16.28** was found to be only about 85 per cent.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of Mardelle Huhn in carrying out the analyses.

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REPORT ON COLOR IN BEER AND WORT

SPECTROPHOTOMETRIC AND PHOTOMETRIC COLOR METHODS FOR BEER

By B. H. NISSEN (Anheuser-Busch, Inc., St. Louis, Mo.),
Associate Referee

The following methods were studied collaboratively.[1]

Definitions applicable to both methods

(1) Beer color intensity shall be defined as ten times the optical density (−log T) at 430 millimicrons wave length, measured in monochromatic light, of a sample of ½ inch thickness, which has the spectral color characteristics of an average beer and is free of turbidity.

(2) "Monochromatic light," for the purpose of the above definition, is a spectral band not to exceed one millimicron in width.

(3) The "spectral color characteristics of an average beer" are such that the ratios of the optical densities at stated wave lengths to the optical density at a wave length of 430 millimicrons are within the range of the standard deviations of the values in Table 1.[2]

TABLE 1.—*Optical density ratios of 38 beers*

(Absorption Spectrum of an Average Beer)

	OPTICAL DENSITY RATIOS (O.D. 430=1.000)						
	400 mμ	410 mμ	420 mμ	430 mμ	440 mμ	450 mμ	460 mμ
Average Ratios	1.8697	1.4471	1.1823	1.000	0.8666	0.7600	0.6644
Standard Dev.	0.0901	0.0392	0.0174	—	0.0096	0.0124	0.0159
	480 mμ	500 mμ	520 mμ	560 mμ	600 mμ	650 mμ	700 mμ
Average Ratios	0.5023	0.3700	0.2766	0.1621	0.0993	0.0540	0.0309
Standard Dev.	0.0192	0.0221	0.0238	0.0226	0.0180	0.0119	0.0078

[1] For results of collaborative work and discussion of the methods, see Proceedings American Soc. Brewing Chemists, 1950.
[2] *Proc. Am. Soc. Brew. Chemists* (1949), p. 140.

(4) The term "free of turbidity" means that the optical density of the beer sample at a wave length of 700 millimicrons in ½ inch thickness is equal to or less than 0.039 times the optical density in ½ inch thickness at 430 millimicrons.

Details of the spectrophotometric and photometric color methods, first action, for beer, the beer calibration method, and the potassium dichromate calibration method are given in *This Journal*, **34**, 61–63 (1951).

RECOMMENDATIONS*

It is recommended—

(1) That the methods described in the report of the Associate Referee on Color in Beer and Wort for 1950, for the spectrophotometric and photometric determination of color in beer, be adopted, first action.

(2) That a beer turbidity method commensurate and compatible with the standard reference color method (SRC) be studied.

(3) That methods either of removing or compensating for turbidity in samples for color measurement and for color in samples for turbidity measurement be studied.

The contributed paper, entitled "The Determination of Alcohol in Wines and Liqueurs," by A. D. Etienne and G. F. Beyer, was published in *This Journal*, **33**, 1016 (1950).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

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

At the 1949 meeting of the Association, C. F. Bruening presented a method for the determination of propylene glycol in vanilla extracts.[1] As this solvent is being used commercially in vanilla and other flavors, the Referee feels that this method should be subjected to collaborative study and that its application should be extended, if possible, to other types of flavors that may contain this solvent.

The Associate Referee on Vanilla Extract and Imitations is reporting a collaborative study of the photometric methods for vanillin[2] and coumarin[3] in imitation vanilla and expects to apply the methods to

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* For report of Committee D and action of the Association, see *This Journal*, **34**, 52 (1951).

[1] *This Journal*, **33**, 103 (1950).

[2] *Ibid.*, **25**, 155 (1942).

[3] *Ibid.*, **22**, 392 (1939).

true vanilla extracts next year. The Referee concurs in the recommendations of the Associate Referee.

No other Associate Referee has submitted a report at this time.

RECOMMENDATIONS*

It is recommended—

(1) That the photometric method for vanillin in imitation vanilla, as given in the report of the Associate Referee on Vanilla Extract and Imitations, be adopted as first action.

(2) That the photometric method for coumarin in imitation vanilla, as given in the report of the Associate Referee on Vanilla Extract and Imitations be adopted, as first action.

(3) That the study of the photometric methods for vanillin and coumarin be continued, particularly in regard to their application to true vanilla extracts.

(4) That the method for determination of propylene glycol in vanilla extracts, *This Journal*, **33**, 103 (1950), be studied collaboratively.

(5) That collaborative studies be continued on the following:

(a) Reflux method for peel oil in citrus fruit juices.

(b) Beta ionone where small amounts are present.

(c) Method for isopropyl alcohol in the presence of acetone.

(d) Method for oil in emulsion flavors.

(e) Maple flavor concentrates and imitations.

No report was given on diacetyl in flavors and nonalcoholic beverages.

REPORT ON VANILLA EXTRACTS AND IMITATIONS

COLORIMETRIC DETERMINATION OF VANILLIN AND COUMARIN

By LUTHER G. ENSMINGER (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

I. INTRODUCTION

Preliminary work has been done on colorimetric methods for the determination of coumarin and vanillin in imitation vanilla flavors by the writer, using the neutral wedge photometer. The methods finally adopted for collaborative study are modifications of those developed and suggested by John B. Wilson, Food and Drug Administration, Federal Security Agency. The preliminary and collaborative studies for vanillin and coumarin are presented separately below (Sections III and IV).

II. PREPARATION OF COLLABORATIVE SAMPLES

The formula for a commercial imitation vanilla flavor was obtained,

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 55 (1951).

and three collaborative samples were made containing the same ingredients qualitatively. The quantitative formulae used for the samples are as follows:

Sample Number	(*1*)	(*2*)	(*3*)
Vanillin, g/100 ml	0.6002	1.0017	0.2000
Coumarin, g/100 ml	0.1824	0.1304	0.1553
Ethyl Alcohol, per cent by vol.	3.0	3.0	3.0
Caramel, per cent	1.0	1.0	2.0
Sugar (Cane), per cent	12.5	12.5	12.5
Tap water to make—	100 ml	100 ml	100 ml

Each sample was prepared in the following manner. The proper amounts of vanillin and coumarin were added to a 1-liter flask and dissolved in the alcohol. Caramel and sugar were dissolved in warm water (about 500 ml) and later added to the flask after cooling. Tap water was then added to make 1000 ml. After thoroughly mixing the ingredients 10 ml of sample was poured into each of 18 bottles in succession and repeated twice more to make 30 ml in each 1 oz. bottle. Samples were sent to 16 collaborators.

III. COLORIMETRIC DETERMINATION OF VANILLIN IN IMITATION VANILLA FLAVOR

A. Preliminary Studies

Two commercial imitation vanilla flavors were purchased and six were prepared in the laboratory, to which sample numbers from I to VIII were given. The colorimetric method for vanillin (*This Journal*, **25**, 155 (1942)) was tried, using 61 filter in the neutral wedge photometer.

On known samples, recoveries of 91.7% to 60.0% were obtained, with the lowest recovery being on the sample containing the greatest concentration of vanillin. Somewhat higher recoveries (about 3% more) were obtained when the standards were run through the complete procedure identical with that for the samples. These facts indicated that vanillin

TABLE 1.—*Recovery of vanillin on samples I to VIII*

SAMPLE NUMBER	ACTUAL CONTENT	RECOVERY	
	(*g/100 ml*)	(*g/100 ml*)	*per cent*
I	—	0.494	—
II	—	0.220	—
III	0.3642	0.375	103.0
IV	0.9493	0.921	97.0
V	1.0497	1.038	98.9
VI	0.7074	0.718	101.5
VII	0.6002	0.588	98.0
VIII	1.0017	1.003	100.1

was lost during the clarifying and filtering processes. And in the higher concentrations more vanillin was lost by the samples than by the standards.

Another series of tests were made to determine the proper aliquots of sample to be added to the 250-ml clarifying flask and of the clarified solution to be put into the 100-ml color-development flask, as well as the amount of lead acetate solution to be added to the clarifying flask. It was found that 2 ml of original sample, 2 ml of lead acetate solution, and 10 ml of filtrate for color development were best. In all filtrations, the first cloudy filtrates were discarded (usually about 10 ml) to prevent later interference. Recoveries of 97.0% to 103.0% for an average of

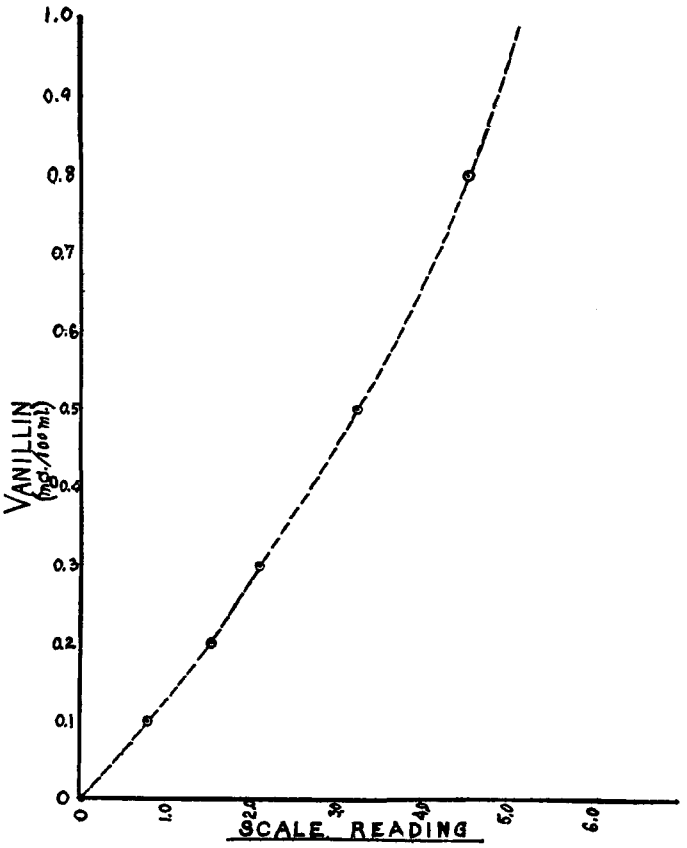

FIG. 1.—Standard curve for vanillin. Prepared May 24, 1950, by Ensminger using 61 filter, 1" cell, and neutral wedge photometer.

99.8% were obtained on the six samples prepared in the laboratory. See Table 1 on page 331 for the complete results.

The standard curve for vanillin must be drawn carefully since it is not a straight line. To indicate its nature, the standard curve used to obtain results in Table 1 is shown in Figure 1.

B. Collaborative Procedure for Colorimetric Determination of Vanillin

Minor changes which simplify or improve the collaborative method have been incorporated in the revised procedure. Details of the method are published in *This Journal*, **34**, 72 (1951).

C. Results of Collaborators on Determination of Vanillin

Results from 12 collaborators were received. Results from another collaborator were received, but in each case his concentration of vanillin was only 85 per cent of the actual amount, and the collaborator stated that he found his vanillin used for the standards to be impure. His results are therefore not considered. Two collaborators used the Klett Colorimeter, while all others listed used the Neutral Wedge Photometer. Many results were received, all determinations are treated alike statistically, and concentrations are given to the second decimal to be consistent with the general ability of various chemists in reading the Wedge photometer. See Table 2 for results on vanillin received from the collaborators. Table 3 on page 335 gives a statistical analysis of results in Table 2.

On examining Table 2 it is noted that only one collaborator is consistently high in recovery of vanillin for all three samples. The writer requested that readings on the photometer be submitted for the purpose of examining them to see that the standard curve was constructed properly. Also, a warning was given in the collaborative procedure that the curve did not obey Beer's Law. Yet, some collaborators attempted to straighten the curve, which made several of the results off further than they should be. Figure 1 is the writer's standard curve for vanillin drawn with a French curve. The above explains why a few chemists were inconsistent among the samples, especially for the highest and lowest concentrations of vanillin.

All concentrations in Table 2 are listed to the second place only, for some chemists cannot compare colors and read results as closely as others, and the concentrations have much less significance to the third decimal point. The writer has checked himself rather closely to the third decimal in vanillin analysis.

Occasionally, a chemist did not check himself on duplicate determinations, and this is probably due to some cloudiness in the filtrates. The method as written above has been tested and corrected for the faults found in collaborative study.

TABLE 2.—*Determination of vanillin in collaborative samples*

CHEMIST	VANILLIN (GRAMS/100 ML)		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	0.60	0.98	0.21
	0.59	0.98	0.20
2[a]	0.60	0.97	0.21
	—	—	—
3	0.60	1.00	0.22
	0.61	1.00	0.22
4	0.62	0.92	0.22
	0.62	0.94	0.22
5	0.59	0.95	0.21
	0.59	0.96	0.20
6	0.58	0.93	0.24
	0.58	0.93	0.23
7	0.63	1.05	0.25
	0.63	1.05	0.25
8[b]	0.59	1.00	0.19
	0.59	1.00	0.25
9[b]	0.61	1.04	0.20
	0.64	1.01	0.19
10[a]	0.61	1.10	0.19
	—	—	—
11	0.54	1.00	0.20
	0.59	0.90	0.21
12[c]	—	—	—
	—	—	—
13	0.65	0.98	0.20
	0.63	1.00	0.23

[a] Only one determination per sample was made.
[b] The Klett Colorimeter was used instead of the Clifford Neutral Wedge Photometer.
[c] The analyst had impure vanillin, and results were 85% of the actual amount for each sample.

In Table 3 the average recoveries of vanillin vary from 98.6% for Sample 2 to 107.5% for Sample 3. The latter is exaggerated because of the low concentration, which also accounts for the highest coefficient of variation. Sample 2 has the highest standard deviation, probable error for a single determination, and probable error of the arithmetic mean, for the

absolute variation of the determinations was greatest at the highest concentration of vanillin. Yet, the true mean representative of the method has a 50% chance of falling within 0.986 ± 0.007, and considering even the lowest value the recovery would be about 98%. Hence, the true mean represented by the method at the greatest point of variance is very close to the actual concentration of vanillin, 1.002 g/100 ml.

The frequency distributions for all these samples are normal, and all results listed in Table 2 are within the permitted ranges, as determined by the arithmetic mean plus or minus 4 times the probable error of a single determination.

The method is not limited to using the neutral wedge photometer or Klett Colorimeter, but more accurate measurements can naturally be

TABLE 3.—*Analyses of collaborative results, vanillin, table 2*[a]

(1) SAMPLE NUMBER	(2) ARITHMETIC MEAN	(3) AVERAGE RECOVERY (%)	(4) STANDARD DEVIATION	(5) PROBABLE ERROR, SINGLE DETERMINATION	(6) PROBABLE ERROR OF ARITHMETIC MEAN	(7) COEFFICIENT OF VARIATION	(8) RANGE	(9[b]) RANGE PERMITTED
1	0.604	100.6	0.024	0.016	0.003	3.97	0.54–0.65	0.54–0.66
2	0.986	98.6	0.047	0.032	0.007	4.77	0.90–1.10	0.86–1.12
3	0.215	107.5	0.020	0.013	0.003	9.31	0.19–0.25	0.17–0.27

[a] Data in columns 2, 4, 5, 6, 8, and 9 are in terms of grams per 100 ml. of sample.
[b] Calculated on basis of arithmetic mean plus or minus 4 times the probable error, single determination.

made on the Beckman or Coleman spectrophotometers if the instruments are available.

In summarizing, the above colorimetric method for vanillin is fairly accurate over a wide range of concentrations, is simple, and is quick. Close checks on duplicates are obtained when care is taken in the procedure, and accuracy is enhanced by running the standards simultaneously with the samples.

IV. COLORIMETRIC DETERMINATION OF COUMARIN IN IMITATION VANILLA FLAVOR

A. Preliminary Studies

The same 8 samples prepared for the preliminary studies of vanillin were also used for the same purpose with respect to coumarin. The article "Quantitative Determination of Coumarin," a contributed paper by J. B. Wilson, *This Journal*, **22**, 392 (1939), was used as a starting point for colorimetric analysis.

Analyses of the six known samples by the above method gave recoveries of 93.1% to 98.9% with an average of 95.4%. In studying this method, as outlined by Wilson, it was necessary to clarify the standards to obtain a higher recovery. In addition, it was found necessary to filter after the color reaction just before reading to obtain consistent results. The method was then modified to carry the standards through the entire procedure simultaneously with the samples, and to filter solutions just before read-

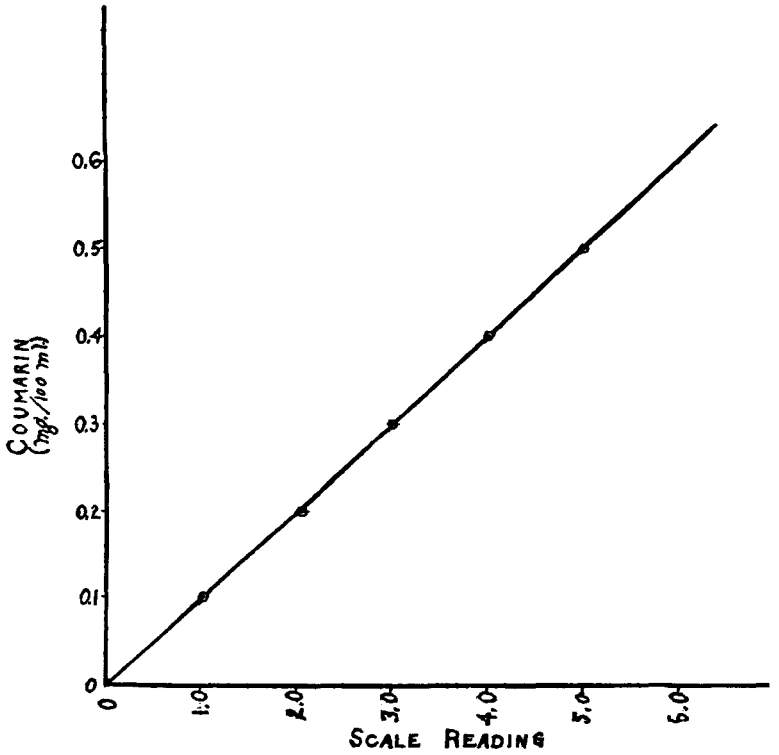

FIG. 2.—Standard curve for coumarin. Prepared May 18, 1950, by Ensminger using 49 filter, ½" cell, and neutral wedge photometer.

ing. An average per cent recovery was then 103.5% on the six samples, the range being 102.5% to 105.3%. The points for the standard curve were not scattered but lay in a very straight line. (See Fig. 2 to compare the coumarin curve with that for vanillin, Fig. 1.)

Table 4 gives the data for the last preliminary study.

As a check, vanillin standards were run through the coumarin procedure to determine whether or not vanillin added to the color intensity. The result was negative.

TABLE 4.—*Recovery of coumarin on samples I to VIII*

SAMPLE NUMBER	ACTUAL CONTENT	RECOVERY	
	(g/100 ml)	*g/100 ml*	*per cent*
I	—	0.214	—
II	—	0.159	—
III	0.2537	0.260	102.5
IV	0.2005	0.207	103.2
V	0.1503	0.155	103.1
VI	0.1342	0.139	103.6
VII	0.1824	0.192	105.3
VIII	0.1334	0.138	103.4

B. Collaborative Procedure for Colorimetric Determination of Coumarin

The method that was used by the collaborators, is published in *This Journal*, **34**, 73 (1951).

C. Results of Collaborators on Determination of Coumarin

Twelve collaborators submitted results with three collaborators giving single determinations and the rest duplicates. One collaborator sent in more accurate determinations, utilizing the Coleman Spectrophotometer, but since the results by the neutral wedge photometer were good the latter were used. All data are rounded off to the second decimal place. A tabulation of results for coumarin from the collaborators is made in Table 5. Table 6 is a statistical analysis of Table 5. Since many results were received, all determinations are treated alike statistically.

A look at Table 5 reveals that results vary very little except for the normal variation to be expected from random error. Sample 3 had an actual concentration of 0.1553 g/100 ml, and in rounding the results off to the second decimal the spread caused by the random error resulted in a greater variation for that sample. The standard curve is a straight line easy to construct, which is more conducive to more uniform results than is a curve not obeying Beer's Law as for vanillin.

Examination of Table 6 shows how well the random error balanced for all samples to give per cent recoveries of 97.6% to 101.0%. The standard deviation, the probable error of a single determination, and the probable error of the mean are so small for the three samples, proving the method to be very consistent. The true mean representative of the method for each sample has a 50% chance of falling between the calculated mean and ± 0.001, which in each case is very close to the actual amount of coumarin present.

Considering the magnitude of each mean, the coefficients of variation are relatively small and uniform. Also, applying the rule that individual results should not deviate from the arithmetic mean by more than 4

TABLE 5.—*Determinations of coumarin in collaborative samples*

CHEMIST	COUMARIN (GRAMS/100 ML)		
	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	0.19	0.13	0.16
	0.19	0.14	0.16
2[a]	0.17	0.12	0.15
	—	—	—
3	0.18	0.12	0.15
	0.17	0.12	0.15
4	0.18	0.13	0.16
	0.18	0.13	0.16
5	0.17	0.13	0.16
	0.17	0.13	0.16
6[b]	—	—	—
	—	—	—
7	0.17	0.13	0.16
	0.17	0.13	0.16
8	0.18	0.13	0.15
	0.18	0.13	0.15
9	0.18	0.13	0.15
	0.18	0.13	0.15
10[a]	0.17	0.13	0.14
	—	—	—
11[c]	0.18	0.13	0.17
	0.18	0.13	0.17
12[a]	0.18	0.13	0.16
	—	—	—
13	0.18	0.13	0.16
	0.18	0.13	0.16

[a] Only one determination per sample was made.
[b] No results.
[c] More accurate results were submitted using the Coleman Spectrophotometer but are not listed.

times the probable error of a single determination, it is seen that no result deviates more than is permitted. And considering the preliminary studies as well as the collaborative, the colorimetric procedure is very consistent over a wide range of concentrations of coumarin without indicating any

TABLE 6.—*Analyses of collaborative results, coumarin (Table 4[a])*

(1) SAMPLE NUMBER	(2) ARITHMETIC MEAN	(3) AVERAGE RECOVERY (%)	(4) STANDARD DEVIATION	(5) PROBABLE ERROR, SINGLE DETERMINATION	(6) PROBABLE ERROR OF ARITHMETIC MEAN	(7) COEFFICIENT OF VARIATION	(8) RANGE	(9[b]) RANGE PERMITTED
1	0.178	97.6	0.007	0.005	0.001	3.93	0.17–0.19	0.16–0.20
2	0.129	98.9	0.004	0.003	0.001	3.10	0.12–0.14	0.12–0.14
3	0.157	101.0	0.008	0.005	0.001	5.10	0.14–0.17	0.14–0.18

[a] Data in columns 2, 4, 5, 6, 8 and 9 are in terms of grams per 100 ml of sample.
[b] Calculated on basis of arithmetic mean plus or minus 4 times the probable error of a single determination.

definite trends of inaccuracy. The method is also quick and relatively easy, and is not limited to the wedge photometer only.

V. PERTINENT COMMENTS BY COLLABORATORS

No collaborator commented about having difficulty with determination of coumarin.

One collaborator reported trouble in obtaining clear filtrates for the vanillin determination, even when filtering through S & S 589 Blue filter paper. (The writer had no difficulty in this respect if, in filtration, the paper is filled full in the beginning to let all pores fill with precipitate and discarding about 10 ml of the first filtrate.) If 10 ml is not adequate, more can be discarded. No cloudy filtrates can be used. The collaborative procedure is altered to indicate a filtrate is to be discarded until clear, rather than limiting the volume to 10 ml.

The analyst who had impure vanillin for the standard suggested that a method be added for purifying vanillin. Purification methods for standards, checks for purity, adjustment of instruments, etc., are found elsewhere.

Other analysts experienced little or no difficulty with either collaborative procedure.

VI. ACKNOWLEDGMENTS

The writer wishes to thank the following collaborators for assistance in the above problems: Shirley M. Walden, Paul M. Sanders, H. C. Van Dame, A. F. Ratay, Sam H. Perlmutter, William A. Bosin, J. E. Roe, Frederick C. Minsher, Angus J. Shingler, R. H. Johnson, D. W. McLaren, and Mathew T. Dow, all from the Food and Drug Administration.

Also, the writer acknowledges the help received in using as starting points the colorimetric methods proposed by John B. Wilson for vanillin and coumarin.

VII. RECOMMENDATIONS*

It is recommended—

(1) That the above colorimetric procedure for the quantitative determination of vanillin in imitation vanilla flavors be adopted first action.

(2) That the above colorimetric procedure for the quantitative determination of coumarin in imitation vanilla flavors be adopted first action.

(3) That further studies be continued on vanilla extracts with the above methods.

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 55 (1951).

MONDAY—AFTERNOON SESSION

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

By MARGARETHE OAKLEY (State Department of Health, Baltimore, Md.), *Referee*

This past year was not a very productive one for the chapter on Preservatives and Artificial Sweeteners. Great effort was put forth to whip the methods into shape for inclusion in the forthcoming *Book of Methods*. Now that work remaining is more in the field of collaborative effort to raise the status of a method from the first action stage to that of official, the Referee's duties become less urgent.

This is particularly true of the work on formaldehyde, where the antiquated methods for its detection were discarded and replaced by more modern, as well as more sensitive ones. The Associate Referee tested the new methods collaboratively and receiving satisfactory results, feels as though the subject is closed and warrants no further time nor effort. The Referee, having tried both the new and the discarded methods, is in full accord with the conclusions of the Associate Referee.

All the work on mold inhibitors has been done on bread, and has been included in the chapter on Cereal Foods. The Associate Referee plans further work on the separation of propionates and acetates in bakery products by chromatographic analysis, as well as sample preparation and preservation. Since these methods have been transferred to cereal products, it is recommended that the whole subject of mold inhibitors be so transferred, leaving only a cross-reference to mold inhibitors in the chapter on Preservatives and Artificial Sweeteners if these substances are used more widely and a method for their estimation is needed in other fields than bakery products.

The Associate Referee on formic acid has reached the conclusion that this substance is no longer used as a preservative. The Referee would concur in a recommendation for its deletion from the chapter on Preservatives and Artificial Sweeteners, except for the fact that the method for estimation of formic acid is so hidden as to be unavailable. It is not listed under formic acid, volatile acids, acid volatile, decomposition, or even under fish—although this is the chapter in which it is printed. Therefore, the Referee is not seconding a recommendation for deletion of the method until there is another, more easily available, in the *Book of Methods*.

Although the method for Monochloracetic Acid has reached a high state of efficiency, the Associate Referee still recommends further collaborative work on beverage bases containing weighting oils, and fruit juices other than orange juice.

There are subjects in the chapter, other than formaldehyde and formic

acid, which need modernizing. It was recommended last year that the qualitative methods for fluorides be studied. Since no one volunteered, the subject was not touched. It is recommended that if no one accepts this associate refereeship before January 1, 1951, the subject be referred to the General Referee on preservatives and artificial sweeteners for investigation.

The subjects of both Thiourea and Quaternary Ammonium Compounds have been quiescent during the past year. This is not serious, since satisfactory methods for both of these have been included in the seventh edition of the *Book of Methods.*

Artificial sweeteners, a large and growing subject, was assigned to one Associate Referee several years ago, when a combined study of saccharin and dulcin was planned, with newer methods for their separation—a major project. Since then, it has been decided that dulcin is more toxic than formerly believed. Since then, also 1-propoxy, 2-amino, 4-nitrobenzene has been developed and found to be toxic, and lately sodium-cyclohexylsulfamate (commercially Cyclamate Sodium) has been approved for sale by the Food and Drug Administration as a non-caloric sweetening agent. The Associate Referee has not been able to put much time on the study, because of a combination of illness and conflicting work. He has tests for the detection of some of the sweeteners which he feels are ready for collaborative work. It is the recommendation of the Referee that, under the circumstances, the person in the Food and Drug Administration now investigating methods for "Cyclamate Sodium" or "Sucaryl" be requested to take an A.O.A.C. associate refereeship in this subject.

RECOMMENDATIONS*

It is recommended—

(1) That further collaborative work be done on monochloroacetic acid on beverage bases containing weighting oils, and on fruit juices other than orange juice.

(2) That collaborative study be continued on quantitative methods for the determination of quaternary ammonium compounds.

(3) That work on formaldehyde be discontinued.

(4) That the subject of mold inhibitors and propionates be transferred to Cereal Foods.

(5) That work on formic acid as a preservative be discontinued and the method (now listed under Fish) be deleted from the chapter on Preservatives.

(6) That studies on qualitative methods for fluorides be continued.

(7) That methods for the detection and determination of "cyclamate sodium" or "sucaryl" be developed.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 55 (1951).

(8) That studies be continued on the Denigès-Tourrou method and the modified LaParola-Mariani methods for the detection of dulcin.

(9) That work be initiated on methods for the detection and estimation of 1-propoxy, 2-amino, 4-nitrobenzene.

REPORT ON QUATERNARY AMMONIUM COMPOUNDS

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

Last year's report described how the Associate Referee took the precaution of supplying collaborators with reagents for determining quaternary ammonium compounds in the collaborative samples of eggs and shrimp so that they would be uniform in all cases. In doing this a 10-gram lot of bromophenol blue was obtained which produced a green instead of a blue color in the ethylene chloride when the quaternary ammonium salt was added and the mixture washed with alkali.

The writer thought that this phenomenon might be due to the presence of some impurity which could be removed, and proceeded on that basis. It was found that such was the case and the procedure given below was used to remove the impurity and a product was obtained which gave a pure blue solution when the test was applied.

TEST FOR PURITY

Place 20 mg of bromophenol blue in a 125-ml separatory funnel, add 50 ml of ethylene chloride, 5 ml of 1% Na_2CO_3 and shake until dissolved. Let stand until the mixture separates into two layers and observe. The lower layer should be colorless, the upper purple. Now add 10 ml of soln containing 0.1 mg of D.C. 12, or other quaternary ammonium compound, again shake, and allow the mixture to settle. The lower layer should have a clear blue color. Draw off the lower layer and examine in a photometer. The absorption should be greatest at about 608 mμ. Compare the absorption curve with that given in *This Journal*, 29, 315 (1946). If the test gives a yellow or green color or if the absorption curve is essentially different from that referred to above, purify as directed below.

PURIFICATION OF SOLID BROMOPHENOL BLUE FOR REAGENT USE

Place 2 g of solid bromophenol blue in a 400-ml beaker and dissolve in 25 ml of 1% Na_2CO_3. Transfer to a 1-liter separatory funnel using about 300 ml of H_2O. Add 500 ml of ethylene chloride and shake. Add 1 ml of a soln containing 10 mg of D.C. 12, or other quaternary ammonium compound, and shake until thoroly extracted. If the lower layer is yellow, repeat the addition of the D.C. 12 soln in 1-ml portions with shaking, until upon separation of the two layers the bottom one has a greenish tint. Draw off the lower layer and discard. Now add to the separatory funnel 200 ml of ethylene chloride and 1 ml of the D.C. 12 soln and shake. This time the lower layer should have a clear blue color. If that layer is green, draw off and repeat addition of ethylene chloride and D.C. 12 until a blue soln is obtained. Wash the water layer with 100 ml portions of ethylene chloride until the lower layer is colorless or only a faint blue. Now acidify the water layer with HCl and extract the yellow precipitate with ethylene chloride until the water soln has only a faint

yellow color. Distil off most of the ethylene chloride and permit the remainder to evaporate spontaneously in a beaker. Grind the residual powder. Test a portion for purity as directed above and, if suitable, use as a reagent.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative study be continued on the following methods:

(a) Method for Fruit Juices, *This Journal*, 29, 318 (1946).

(b) Shorter Method for Fruit Juices, *Ibid.*, 29, 319.

(c) Method for Bottled Sodas, *Ibid.*, 29, 323, subject to increasing the volume of bromophenol blue reagent to 5–10 ml.

(d) Method for Milk, *Ibid.*, 29, 324, on samples containing preservative quantities of quaternary ammonium compounds.

(e) Method for Mayonnaise, Salad Dressings, and Sandwich Spreads, *Ibid.*, 29, 323.

(f) Method for Pickles and Relishes, *Ibid.*, 29, 326.

(2) That work be continued on the determination of quaternary ammonium compounds in shrimp.

REPORT ON MONOCHLOROACETIC ACID

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

The official methods for monochloroacetic acid in beverages have frequently been applied to beverage bases, and from time to time the results obtained on citrus bases have disturbed analysts, because, while considerable quantities of halogen may be obtained by the quantitative method, the sample gives no test for monochloroacetic acid when none of this preservative is present. This condition results from the presence of brominated olive oil used as a weighting oil.

The low density of the citrus oils (about 0.85) causes them to settle out in the neck of the bottle of beverage which has a much higher density (about 1.04). To counteract this tendency, a sufficient quantity of brominated olive oil is added to the citrus oil to raise the density of the mixture to approximate that of the beverage. When emulsified in the base and diluted into a beverage, such a mixture seldom yields a ring in the bottle neck and is more easily redistributed throughout the contents of the bottle by inverting it.

In the paper "Determination of Monochloroacetic Acid in Beverages," *This Journal*, 25, 145 (1942), it was shown that as far as the analysis of beverages is concerned the brominated olive oil has little, if any, effect upon the determination of monochloroacetic acid. Bottled sodas contain 12 to 24 mg of brominated olive oil which corresponds to from 0.4 to 0.7

* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 55 (1951).

mg of monochloroacetic acid per bottle as calculated from the yield of halogen obtained when applying the method. However, in the case of the beverage base enough halogen may be obtained to be confusing.

In the case of a beverage base which is to be diluted at the rate of 1 or 2 fl. oz. to a gallon or more of beverage, the brominated olive oil may cause an apparent content of monochloroacetic acid to approximate preservative quantities. The writer has in mind a case where the analysis of a beverage base gave an apparent monochloroacetic acid content of 500 p.p.m., while neither the indigo test nor the pyridine test was positive.

Since in this case the quantitative procedure is at fault, the following modified method is suggested for study.

DETERMINATION OF MONOCHLOROACETIC ACID IN BEVERAGE BASES CONTAINING HALOGENATED WEIGHTING OILS

Place 100 ml of sample in a continuous extractor suitable for extracting 200 ml of liquid, add 100 ml of H_2O containing 7 ml of H_2SO_4, and extract for 2–3 hrs. with ether. (Use the length of time found necessary for a recovery of at least 95% when known quantities of monochloroacetic acid are extracted in the apparatus.) Transfer the ether soln to a separatory funnel; add 10 ml of 1 *N* $NaHCO_3$ soln, and shake for 5 min. Test the water layer with *p*H paper. If acid, add 5 ml $NaHCO_3$ soln and again shake. When the *p*H paper indicates a *p*H of about 8, separate the water layer. Add 50 ml of 1 *N* NaOH, hydrolyze, and determine halogen as in the official method.

RECOMMENDATIONS*

It is recommended—

(1) That the method for determination of monochloroacetic acid in beverage bases containing halogenated weighting oils be studied collaboratively.

(2) That further work be done on the determination of monochloroacetic acid in fruit juices other than orange juice.

The report on Acetic and Propionic Acids in Bread (Mold Inhibitors, Propionates) was transferred to the chapter on Cereal Foods, page 284.

REPORT ON EGGS AND EGG PRODUCTS

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

Subcommittee C recommended that further work be done to develop a method for the determination of added glycerol in egg mixtures containing added sugars. It was also recommended that the method proposed last year by the Associate Referee for the determination of ammonia nitrogen in eggs be studied collaboratively.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 55 (1951).

GLYCEROL IN EGGS WITH ADDED SUGARS

The Associate Referee reports that he has studied several methods which may be applicable to this problem, but he has been unable to devise a method this year which he feels is good enough to submit for collaborative study. The Referee recommends* that the work on this problem be continued.

AMMONIA NITROGEN

The Associate Referee reports that he submitted for collaborative study the method proposed in his 1949 report. The results received from five collaborators covering four different samples of eggs indicate that the method needs further study. The Associate Referee recommends* that the work be continued, and the Referee concurs in this recommendation.

REPORT ON FREE AMMONIA IN EGGS

By ELIAS B. BOYCE (State Dept. of Public Health, Boston 33, Mass.), *Associate Referee*

PREPARATION OF SAMPLES

The Associate Referee prepared samples of eggs for collaborative determination of free ammonia by breaking out 12 dozen eggs of reasonably good quality and mixing the broken out eggs in a six liter flask. Each dozen broken out eggs was "blended" for about a minute in a Waring Blendor and then transferred to the 6 liter flask. The flask was permitted to stand at room temperature during the working hours over a period of four days, a portion being removed each day and frozen in a large ice cube tray in a refrigerator. The portion remaining on the fourth day had a faint "sweet" odor suggestive of incipient decay.

The frozen eggs in each tray were then divided vertically in 5 blocks for distribution to collaborators. The frozen blocks of egg were shipped or delivered to collaborators packed in a container in dry ice in a frozen condition with directions to thaw out immediately before the determination of free ammonia was to be made. One of the sets of samples was reserved for analysis in the laboratory of the Associate Referee.

The method of analysis is found in the 1949 report of the Associate Referee for determination of free ammonia in eggs.[1]

For convenience of the collaborators portions of the antifoam reagent and of standard ammonium sulphate solution were sent out with each set of samples.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 49 (1951).
[1] *This Journal*, 33, 703 (1950).

RESULTS

The results of the analysis of the (4) sets of samples are shown in Table 1.

TABLE 1.—*Analytical results*

SAMPLE NO.	LABORATORY NUMBER NH_3 MG/100 GM				
	1	2	3	4	5
1	1.35	1.12	0.56	1.49	0.95
2	1.00	1.43	0.64	1.52	1.32
3	1.30	1.42	1.12	1.60	1.22
4	3.00	3.04	2.40	3.32	2.49

COMMENTS OF COLLABORATORS

"The samples arrived frozen with excess of dry ice in the carton. The procedure as forwarded was followed using 20 gram samples. Excellent agreement was experienced between duplicates." (*Gilman K. Crowell*)

"The last paragraph in the directions appears slightly confusing especially as to the determination of the reagent blank. The blank was determined by adding 0.4 mg of NH_3 to the reagent black distillate and comparing the nesslerized solution with the standard containing 0.4 mg of NH_3, the difference between the two being taken as the reagent blank. However this gave a negative blank of about .01 mg NH_3 so it was ignored in calculating the NH_3 in the samples of frozen eggs." (*Sidney Williams*)

DISCUSSION

The results of this collaborative study clearly show that further work needs to be done before the procedure can be recommended as a standard method.

RECOMMENDATIONS*

It is recommended—

(1) That work on the method be continued.

(2) That investigation be conducted to ascertain the effect of freezing of the sample and of the amount of beating upon the results obtained.

(3) That further study be made of the relationship between the rate of aeration and the completeness of removal of the free ammonia.

(4) That the procedure be revised as indicated by this further study.

ACKNOWLEDGMENT

The Associate Referee gratefully acknowledges the assistance of the following collaborators for their help and comment.

Gilman K. Crowell, State Dept. of Health, Concord, N.H.; George Edwards, Mass. Dept of Public Health, Westfield, Mass.; C. E. Hynds, Dept. of Agriculture & Markets, State of New York; Sidney Williams, Federal Security Agency, U. S. Food and Drug Administration, Boston, Mass.

* For report of Subcommittee C and action of the Association, see *This Journal*, **34**, 49 (1951).

REPORT ON EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Associate Referee reports this year are limited to those on Beverages; Drugs, Spices and Miscellaneous Materials; and Dairy and Egg Products.

The report on extraneous matter in beverage materials discusses two procedures. The procedure for making a mold count on pineapple juice is simply an adaptation of the well-established mold count in citrus juices, and the Referee concurs in the recommendation* that it be adopted, first action. The method for mold in crushed pineapple should be studied collaboratively.

The report on extraneous materials in drugs, spices, and miscellaneous products suggests a modification for extraneous matter in onion powder **(35.85)**. This method has already been made first action, and it is recommended* that the suggestion be subjected to collaborative study, so that a decision on the status of the present method and revision can be made as soon as possible.

The report on extraneous materials in dairy products and eggs summarizes old and discusses new techniques to be applied to the detection of dung in cheese. It is recommended* that this work be subjected to collaborative study at an early date.

REPORT ON EXTRANEOUS MATTER IN BEVERAGE MATERIALS

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

A method for the examination of mold in crushed pineapple has been developed by (Mrs.) M. G. Yakowitz and W. V. Eisenberg of the Microanalytical Branch of the Division of Microbiology.

The method for mold count in citrus juices (**42.5**, 6th Ed., *Methods of Analysis*), has been adopted to use on pineapple juice by providing for the addition of 0.5 ml conc HCl (for the purpose of dissolving the calcium oxalate needle crystals) before making the volume to 15 ml with 3% pectin solution. Also the volume (in ml) of sediment in the centrifuge tube should be recorded.

It is recommended that a method for mold in crushed pineapple be adopted and studied collaboratively during the coming year. On the

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 54 (1951).

basis of the work done on tomato products, berries, small fruits, and citrus juices it is recommended* that the mold count procedure for pineapple juice be adopted, first action.

The method for mold in crushed pineapple is as follows:

Drain contents of can on 8–12 inch No. 8 sieve for 2 min and weigh. Use 8-inch sieve for less than 3 lbs of product and 12 inch sieve for over 3 lbs.

Examine the drained juice by method 35.5, modified by adding 0.5 ml conc HCl (to dissolve calcium oxalate needle crystals) before making the volume to 15 ml with 3% pectin soln. Read volume of sediment in centrifuge tube. In addition to checking microscopic fields as positive in the count, also indicate those fields which are positive due to Oospora mold.

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

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

A revision of the method for extraneous matter in onion powder, 35.85, has been recommended by the Associate Referee.*

Rodent hairs have a tendency to cling to the plant tissues in finely ground onion and garlic powders, and a better recovery of rodent hair fragments was obtained by changing method 35.85 as follows:

LIGHT AND HEAVY FILTH

Use 25 g of the well-mixed sample and proceed for the determination of heavy filth as in Method 35.85.

Draw air thru the plant tissue of the Büchner funnel until the solvent is evaporated. Transfer to a 1-liter Wildman trap flask. Add 10 ml of 10% sodium oleate soln. Add 200 ml of 40% alcohol. Boil one min. Cool to 25°C. Add 20 ml gasoline. Stir. Add 40% alcohol and let stand 30 min, stirring every five min. Trap off and filter on paper.

Using mineral oil to examine the filter paper microscopically. Make a second extraction.

METHOD FOR POST-MILLING INFESTATION

Use a 100 g sample. Add 10 ml of 10% sodium oleate soln. Add 400 ml of 40% alcohol soln. Boil for one min. Pour thru a 20-mesh sieve. Examine screenings macroscopically for insects, large fragments, and long hairs.

Put washings in a 2-liter Wildman flask and float with 40 ml gasoline. Proceed as in above method.

Examine paper microscopically.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 54 (1951).

REPORT ON EXTRANEOUS MATERIALS IN DAIRY PRODUCTS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

Feed for milk-producing animals consists of forage supplemented by concentrated feed mixtures. The forage may be moist green meadow grasses, soiling crops and silage, or dried forage as in hays, straws, and fodders. These forage feeds have a high crude fiber content; on a dry basis, 20 to 40 per cent in hay and up to 50 per cent in straw. The concentrates are the by-products from the milling industry; bran shorts or middlings, mill run products; meals from the expression of oil such as cottonseed, linseed, and soybean, or by-products of cheese and butter factories, meat and fish packing industries; and grains, whole or fragments.

The digestible nutrients in these feeds are proteins, carbohydrates, fats, and mineral constituents. Much of the crude fiber or roughage, which is largely composed of lignin and mineralized cellulose, is not digested and is eliminated in the dung.

The nutritive elements are in the plant cells. The plant cell consists of a cell wall composed of polysaccharides, cellulose, hemicelluloses, and pectins. With these are associated other substances, lignin, suberin, and cutin. Suberin and cutin are varying mixtures of organic acids present in part in the form of fats. Cell walls may also contain mineral substances such as silica, calcium carbonate, and calcium oxalate, as well as more complex organic compounds including tannins, oils, and resins.

The cell contents consist of cytoplasm, a more or less viscous fluid; plastids, nucleus, and vacuoles. The plastids are specialized for carbohydrate synthesis and transformation. They contain the visible pigments in the chromoplasts; the green chlorophyll is most predominant in plants. With chlorophyll are associated the yellow carotinoid pigments carotin and xanthophyll. The vacuoles contain a viscous fluid which is homogenous in appearance. It consists of water with a variety of substances in molecular and colloidal solution. The vacuole is a reservoir of nutritive materials: minerals, organic acids, alcohols, carbohydrates, slimes, glucosides, anthocyanin; and soluble yellow pigments, amides, alkaloids, albumins, enzymes, tannins, and aromatic compounds. These soluble substances may become solid and crystallize or precipitate.

It is the undissolved nutritive elements that are seen in undigested plant fragments. The most conspicuous visible carbohydrates are starch and cellulose. Other visible nutritive bodies are the crystalline or noncrystalline proteins. The best known are the albumin crystals in the aleurone grains. In maize and wheat kernels they lie in the outermost layer of endosperm cells. Visible fats and oils occur emulsified with water as droplets in the cytoplasm. Crystals may lie in the cytoplasm, vacuoles,

or occasionally in the nucleus, or they may be imbedded in the cell walls. Calcium oxalate is the most prevalent.

The digestive juices of the animal remove these nutritive elements from the ingested chewed plant fragments. The cell walls may also be digested to a greater or lesser degree as well as the lamellae or cell plates between adjacent cell walls which are composed of cellulose and related carbohydrates such as pectin. When the lamellae are digested, the cell walls are separated and the plant tissue disintegrates easily in the dung fragments.

Since the cells comprising the dung fragments are deprived of the nutritive elements, they will be very clear and translucent, sometimes transparent. Chemically, lignin requires the action of hydrolytic agents at high temperatures and pressures to liberate the cellulose.

This lignified and mineralized cellulose is found in the thickened walls of plant sclerenchyma tissue, such as stone cells; in the fibrovascular bundles, the cross cells and other cereal seed coat layers, and plant hairs. The stems and the empty and flowering glumes and palets of cereals (chaff) have epidermal cells which are strongly silicified and resist digestion. Hence, the stems and stalks of the grasses and the veins of leaves composed of vascular bundles may not be completely digested. The parenchyma of the leaves contains the greater part of the available food elements.

The fiber of immature hay and of root crops such as sugar beets, mangel, and turnips is digestible.

In a well-nourished animal, the plant fragments may not be entirely digested and some reserve elements may remain. In the excrement of an animal given an insufficient diet, the digestion of the feed is extreme and even the ligneous parts may appear corroded.

Thus dung fragments are frayed and jagged from chewing and fermentation, the nutritive cell contents are gone and the walls are partially digested. The normal undigested plant fragments have clean cut fractures, the cell walls are intact and the nutritive elements remain. .

Another characteristic of dung is the heterogeneous mixture of the various elements of the forage. The undigested plant fragments in cheese and other dairy products are clean and isolated. Dry forage that can be easily scattered such as bran, glumes, meal, hay and straw may fall into the milk. These will be undigested and will contain the nutritive elements. The digested dung fragments may be massed together in a confused aggregate of various types of forage debris with many free spirals from the conductive vessels evident.

The dirty, brownish appearance of the dung fragments and the brown specks often referred to, are usually due to mucilaginous substance which is found in large quantities in the dung of cows, sheep and goats. The excreta pellets of goats and sheep are almost spherical in shape, the digested

plant fragments being bound together with this mucilage, which forms a thick coating on the surface, dark brown in appearance.

Repeated tests on authentic material show that this brown amorphous mucilage on the fragments withstands all of the chemical treatment normally applied to cheese, and it remains on the dung fragments as they are filtered out on the filter paper for microscopic examination. Some of the mucilage may be in the jagged ends or in the crevices of the dung fragments. It may be found binding several digested fragments together in small clumps. The mucilage is insoluble in alcohol, formaldehyde, weak acids, weak alkalis, chloral hydrate, and is not affected by pancreatin digestion.

Various methods of staining to differentiate digested from undigested plant fragments and to obtain a differential stain for the mucilage were tried. Methylene blue alone or in combination with other stains colored

FIG. 1.—Portion of sheep dung showing translucent digested plant fragments, free spirals, jagged ends of fragments, held together with mucilage.

the mucilage green. This material resembled chlorophyll to some extent and therefore was discarded. A good contrast was obtained by staining the plant fragments with 2½ per cent solution of safranin in 95% alcohol. Elements of the plant tissue are stained a deep pink while the mucilage is unstained and appears as a granular or mottled light brown coating on the surface, in the crevices or ends of the dung fragments.

Safranin stains lignified, cutinized, and suberized cell walls, chitin, and the nucleus, proteins and chromosomes. Undigested plant fragments will therefore be deeply stained with safranin. The cell walls and some of the nutritive reserve elements will take the stain and will be sharply defined microscopically. The cells of the undigested plant fragments retain their normal outline and contents.

Since the dung contains large quantities of stem tissue and straw which are composed mostly of the lignified and mineralized cellulose not digested by the animal, they will also stain with safranin. However, some or all of their nutritive elements have been digested so, in general, they are less brilliantly stained and they will appear dirty with mucilage on their surface. They may be eroded and macerated. The stained cell walls and bundles will be sharply defined.

The mucilage is precipitated by the alcohol in the wetting mixture used for examining the filter paper. It can be seen at lower power but it is seen much better at a higher magnification.

METHOD

Cover the residue from 225 g of cheese on a fine fritted funnel with a soln of 90 ml of 95% alcohol and 10 ml of formaldehyde. Allow to stand 2 min, then drain.

Cover the material with 95% alcohol. Add 3–4 drops of 2½% safranin in 95% alcohol, let stand for 10 min, then drain and wash with 95% alcohol until the drops of alcohol are colorless. Wash thoroly with water. Cover the residue with Hertwig's soln (19 ml HCl, 60 ml glycerine, 270 g chloral hydrate, 150 ml water). Let stand for 1 min. Wash with water, thoroly. Transfer to a filter paper. Wet the paper with a 1:1 mixture of 95% alcohol and glycerine. Examine at 60×–70× with the stereoscopic microscope.

A large number of the dung fragments will have some of the mucilaginous substance on the surface, in the ends or in the crevices. The plant fragments will appear to be clean, deeply stained, their normal contours intact, nutritive elements in the cells visible, and will have none of the mucilage on the surface.

Some brown pigmented fragments may be confused at low power with the brownish granular mucilage on the surface of the dung fragments. These should be mounted in a drop of the alcohol-glycerine mixture and examined at high power with the compound microscope. Pigment granules and reserve elements will be inside of the cells, whereas the mucilage is always on the outside.

CHARACTERISTICS OF DUNG AND UNDIGESTED PLANT FRAGMENTS

Dung fragments	*Plant fragments*
Nutritive elements, cell walls, and lamellae digested to a greater or lesser degree.	Cell walls intact. Nutritive elements visible. Appearance normal.
Tissues easily disintegrated.	Tissues intact.
Cells very clear and translucent. Empty of cell contents.	Cells opaque with contents visible.
Walls of cellulose impregnated with lignin and minerals remain. Walls of digested non-lignified cellulose gone.	Walls of pure cellulose and derivatives are all visible.
Ends of fragments, fibrous and jagged.	Ends of fragments clean cut and normal to ends.
Heterogenous mixture of forage debris. Often held together with mucilage.	Plant fragments clean and isolated.
Brown mucilage on surface, in ends or crevices of fragments.	No mucilage on surface.
Lignified walls stained with safranin. Mucilage brown and unstained.	Cell walls, woody elements and nutritive reserves stained deeply.

RECOMMENDATIONS*

It is recommended—

(1) That the staining of plant and dung fragments with a combination universal textile stain be further studied.

(2) That samples of cheese containing dung be submitted for collaborative study.

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* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 54 (1951).

REPORT ON MEAT AND MEAT PRODUCTS

By R. M. MEHURIN (Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.), *Referee*

The Associate Referee on Horse Meat in Ground Meat reports collaborative results on the chemical method proposed last year for the detection of horse meat in hamburger. Fairly good results were obtained with the mixtures sent out. The Associate Referee recommends* that further work be done with the method. The Referee concurs.

The Associate Referee on Starch in Meat Products states that he is making progress with a new method but has no report ready for this meeting. He recommends* that work be continued on this subject. The Referee concurs.

The Associate Referee on Creatin in Meat Products has sent out samples to collaborators for the direct determination of creatin. This was done, however, too late to have results ready for this meeting. He recommends* that work be continued during the coming year. The Referee concurs.

No report has been received from the Associate Referee on Soya Flour in Meat Products concerning further work on the two quantitative methods reported by him at the 1947 meeting. The use of this material in sausage is extremely limited in this country and is not permitted in Canada. For this reason, it is difficult to secure an Associate Referee and collaborators who are willing to devote the considerable amount of attention that will be required to work out, if possible, more accurate quantitative methods than those reported by the Associate Referee at the 1947 meeting. It is recommended,* therefore, that further work by the Association on the determination of soya flour in meat products be discontinued for the present.

REPORT ON HORSE MEAT IN GROUND MEAT

By CLAUDE E. HYNDS (Food Laboratory, New York State Department of Agriculture and Markets, Albany 1, N. Y.), *Associate Referee*

Last year the Associate Referee reported that the hexabromide number of the extracted fat and the qualitative test on the lean portion for glycogen were satisfactory methods for the detection of horse meat in ground meat mixtures. This year, test samples of known composition have been submitted to collaborators for determinations, using the methods as described (*This Journal*, **33**, 752).

A quantity of authentic horse meat, appearing to contain an average amount of fat, was run through a meat grinder and well mixed. Also, a

* For report of Subcommittee C and action of the Association, see *This Journal*, **34**, 50 (1951).

quantity of beef appearing to contain about the same amount of fat was treated similarly. No attempt was made to determine the percentage of fat in these meats. Samples of the following composition were submitted to collaborators.

Sample	*Composition*
A	Mixture of horse meat 25% and beef 75% by weight, thoroly mixed and then reground.
B	Mixture of horse meat 50% and beef 50% by weight, thoroly mixed and then reground.
C	Horse meat, 100%.
D	Beef, 100%.

Each sample was divided into five parts, one of which was analyzed by the Associate Referee. Each of the remaining subdivisions was transferred to pint fruit jars and autoclaved at 15 lb. steam pressure for 20 minutes. Autoclaved portions of each unknown sample, and an identified, authentic, autoclaved sample of horse meat, were sent to each of four collaborators together with a copy of the methods. Results follow:

TABLE 1.—*Per cent of horse meat in ground meat*

SAMPLE	HEXABROMIDE NUMBER					TEST FOR GLYCOGEN					LABORATORY 1, ONLY	
	LABORATORY					LABORATORY					N_D^{40} OF FAT	GLYCOGEN BY MODIFIED TEST
	1	2	3	4	5	1	2	3	4	5		
A	27.8	23.6	39.7	26.1	14.0	−	+	−	+	+	1.4595	++
B	52.8	45.2	61.0	42.5	31.6	+	+	?	+	++	1.4600	++
C	86.6	60.1	85.7	59.2	72.5	−	+	?	+	+++	1.4610	++++
D	1.8	1.7	3.3	2.4	0.0	−	−	−	−	−	1.4575	−
Horse meat authentic	130			63.1					+		1.4618	

COMMENTS OF COLLABORATORS

Laboratory 1, Elias B. Boyce, Food and Drug Division, Massachusetts State Department of Health, Boston, Mass.—"Samples A, B, and C are mixtures. Sample D is beef. In the test for glycogen, the addition of 2 vol. of glacial acetic acid precipitates the glycogen so it is left on the filter paper and the filtrate gives no test. Too much I-KI is added, tending to obscure the test. It is more satisfactory to add dropwise, usually 2–3 drops being sufficient. Hexabromide number appears to be well suited to detection of mixtures of relatively small amounts of horse fat."

Laboratory 2, Richard T. Merwin, Connecticut Agricultural Experiment Station, New Haven, Conn.—"I like the hexabromide method's simplicity and directness. However, the positive tests obtained for glycogen did not seem satisfactory and were interpreted as positive only in relation to Sample D. I found after many attempts to develop a satisfactory glycogen test that the following method gave far more sensitive and positive results: After boiling the meat in salt solution and filtering, 10 ml of filtrate is saturated with solid ammonium sulphate and refiltered. The iodine reagent is added to the second filtrate a drop at a time until a wine red color is produced. One ml of the reagent produces a chocolate brown color.

"The ammonium sulphate acts as a starch precipitant, it seems to me, just as well if not better than the acetic acid. It also sensitizes the test. Although it is also a glycogen precipitant, the precipitate, being colloidal, filters through even a Whatman No. 42 paper, so that the glycogen appears in the filtrate to give the test, whereas any vegetable starch remains on the paper."

Laboratory 3, James B. Hunter, Texas State Health Department, Austin, Tex.—No comment.

Laboratory 4, John Bailey, Div. of Foods and Dairies, Illinois Department of Agriculture, Chicago 1, Illinois.—Reports melting points on recovered hexabromide as follows: Sample A, 175–201°C.; Sample B, 176°C.; Sample C, 173°C.; Sample D, none present; and Horse meat, authentic, 173°C.

Laboratory 5, Claude E. Hynds, State Food Laboratory, New York State Department of Agriculture and Markets, Albany, N. Y.—"No difficulties have been experienced in using the glycogen test as described. In fact, it worked so well that it could almost be used as a quantitative colorimetric method. The discrepancy among laboratories may be due to different batches of reagents or to some minor detail not understood."

From the foregoing results and comments, it is recommended* that more work be done and more collaborative samples analyzed.

REPORT ON NUTS AND NUT PRODUCTS

By A. M. HENRY (Food and Drug Administration, Federal Security Agency, 416 Federal Annex, Atlanta 3, Ga.), *Referee*

The methods, *This Journal*, **33**, 85 (1950), proposed and made first action last year, were studied collaboratively on pecans and peanuts this year, which are reported in Table 1. The Referee thanks the following collaborators, who have made this report possible.

E. A. Epps, Jr., Baton Rouge, La.; F. N. McMillan, Tallahassee, Fla.; L. L. Barker, Atlanta, Ga.; R. E. Dickey, Dover, Del.; R. A. Piper, Montgomery, Ala.; Law & Co., Atlanta, Ga., and the following, all of the Food and Drug Administration: M. M. Jackson, Philadelphia, Pa.; H. O. McComber, Baltimore, Md.; F. H. Collins, Cincinnati, Ohio; F. E. Yarnell, Kansas City, Mo.; A. L. Suslam, Boston, Mass.; R. L. Herd, St. Louis, Mo.; L. H. Feldstein, Denver, Colo.; W. B. Tarver, Atlanta, Ga.

In addition, W. B. Tarver of this laboratory applied them to coconut, coconut with added sugar, and chinquapins, which have a high starch content. The results are reported in Table 2. E. M. Hoshall of the Baltimore District, and M. M. Jackson of Philadelphia District, made suggestions for improvement in the wording of the methods. Other collaborators also made valuable suggestions. The methods gave results that are satisfactory.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 50 (1951).

TABLE 1.—*Nuts—general methods—1950*

COLLABORATOR	MOISTURE		ASH		FAT DIRECT		FAT INDIRECT		CRUDE PROTEIN		REDUCING SUGARS		SUCROSE		CRUDE FIBER		SALT (FIXATIVE)	
	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS	PECANS	PEANUTS
1	3.27	4.11	1.73	2.25	68.89	51.68			11.38	29.38					2.18	2.46	0.10	0.10
					68.69	51.65			11.13	30.19					2.10	2.31		
					68.98	52.07			11.21	29.63					2.10			
2	3.33	5.01	1.72	2.05	71.53	50.21			11.25	28.94					2.06	2.64		
3	3.68	4.93	1.69	2.21	70.50				11.12	29.44	0.00	0.00	3.35	2.22				
	3.63	4.89	1.76	2.26					10.44	29.37				3.02				
4	3.65	4.90									0.23	0.17	3.92	3.98			0.04	0.04
	3.60	4.88									0.22	0.16					0.04	0.05
5	3.63	5.00	1.71	2.13			70.59	50.15	10.75	27.52	0.25		4.68	4.34	1.80	2.22	0.03	0.03
	3.60	5.00	1.72	2.12			70.65	50.06	10.50	27.33	0.25		4.54	3.99	1.79	2.21	0.03	0.03
									10.74	27.51								
6	3.97	5.27	1.73	2.08	69.00	48.10			11.10	27.90	0.10	0.12	4.74	4.78	2.98	2.17	0.03	0.02
	3.99	5.29	1.74	2.13	69.30	49.10			10.60	28.10					3.68	2.48		
7	3.16	4.79	1.77	2.34	70.65	50.88	70.04	50.49	11.33	28.71	0.08	0.00	4.15	4.39	1.83	2.30	0.08	0.08
	3.16	4.78	1.77	2.34	70.90	50.68	70.32	50.25	11.44	28.84	0.18	0.00	4.10	4.37	1.90	2.28	0.12	0.08
	3.18	4.77	1.78	2.36	70.96	50.62	70.41	50.17	11.43	28.68							0.11	0.10
8	3.21	4.30	1.74	2.33	71.13	49.96			10.80	27.50					2.00	2.57		
	3.19	4.25	1.75	2.23	71.05	49.80			10.70	27.40					2.01	2.51		
	3.17	4.19	1.75	2.35	71.02	50.26			10.80	27.50					2.00	2.58		
9	3.55	4.86	1.67	2.10	69.99	49.37			12.25	29.50	0.00	0.00	4.05	4.26	3.25	2.85		
	3.54	4.84	1.71	2.10	71.09	48.75			11.75	29.80	0.00	0.00	4.07	4.25	3.27			
10	3.95	5.11	1.91	2.36	69.79	51.03			11.29	27.89					1.87	2.19		
	3.80	5.15	1.89	2.38	69.60	50.98			11.49	27.84					1.85	2.04		
11			1.76	2.13					11.17	28.39					2.19	2.84		
			1.75	2.14					11.10	28.47					2.19	2.96		
										28.30					2.07			
12	3.61	4.91	1.61	1.62					10.25	28.50					3.25	2.68		
	3.52	4.86	1.57	1.56					9.88	27.00					3.14	2.64		
13	3.20	4.60	1.82	2.19	72.45	49.80			11.19	28.88					1.98	2.22		

TABLE 2.—*Nuts—general methods*

COLLABORATOR	MOISTURE		ASH		FAT DIRECT		FAT INDIRECT		CRUDE PROTEIN		REDUCING SUGARS		SUCROSE		CRUDE FIBER		SALT (FIXATIVE)	
SUB	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2
Chinquapins	28.56	28.63	1.50	1.49	8.44	8.93	8.54	9.06	5.58	5.59	3.58	3.59	11.08	11.17		2.50	Trace	
Dried Coconut	3.79	3.72	2.06	1.94	66.75	66.54	66.54	66.71	7.32		0.34		6.34			3.83	Trace	
Coconut with added sugar and salt	1.60	1.63	1.10	0.85	26.31				2.96	2.87	Trace		65.20	69.70	2.53	2.10	0.15	0.16

The procedures for preservation and preparation of samples have been given some study but no improvements can be reported at present. However, editorial clarification is recommended.

No work has been done this year on added starch in peanut butter, **25.13,** and on sorting methods for moisture and fat.

Some work has been done on the determination of glycerol, **25.14,** and propylene glycol in shredded coconut, but the work is not complete.

Some work has been done on the development of free fatty acids in nuts which indicates that some types of rancidity can be correlated with the free fatty acids. The work has not been carried far enough to report definite results.

RECOMMENDATIONS*

It is recommended—

(1) That the methods made first action last year, *This Journal,* **33,** 85 (1950), be made official, with the following editorial changes.

(a) Moisture. Drying with heat:

Dry 2–5 grams of prepared sample (procedure) at 95–100° under constant pressure, not to exceed 100 mm of Hg for ca 5 hours; cool in a desiccator and weigh. Dry again for 1 hr or until loss in weight is not more than 0.1%. Report loss in weight as moisture.

(b) Crude fat: Direct method:

Added sugar interferes with complete extraction of fat; in such cases extract with water first, removing any fat from water with petroleum ether and adding to ether extract. Extract 2–5 g of sample, dried as in **(a),** with anhydrous ether, equal to 27.24 grade, for 16 hours. Dry extract at 95–100° for 30 min. Cool in desiccator and weigh. Dry again for 30 min or until loss in weight is not more than 0.1%. Report loss in weight as crude fat or ether extract.

(e) Ash. See **25.9,** or if added chlorides present, **25.10.**

(f) Substitute for last paragraph:

Proceed as directed under **34.39** or **34.53,** using 50 ml aliquot (representing 4 g of sample) unless added sugar is present when proportionately smaller aliquot should be used. See last paragraph under **(g)** sucrose. Express results as dextrose or invert sugar.

(h) Delete Open Carius Method.

(2) That the following sentence should be inserted between the two sentences in preparation of sample:

(a) *Nuts in shell*—"The skin or spermoderm should be included with the meat in all nuts, including peanuts and coconuts unless specifically excluded by description."

(3) That sorting methods for moisture and fat be studied.

(4) That methods for added starch and other additives in peanut butter be studied.

(5) That methods for added glycerol and propylene glycol in shredded coconut be studied.

(6) That chemical methods for decomposition in nuts be studied.

* For report of Subcommittee C and action of the Association, see *This Journal,* **34,** 50 (1951).

REPORT ON GUMS IN FOODS

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

The definition and standard for cream cheese and Neufchatel cheese as promulgated by the Federal Security Agency[1] permits the use of karaya, tragacanth, and carob bean (locust bean) gums, gelatin, and algin. The Associate Referee on gums in cheese has refined the current method[2] for detection of karaya, tragacanth, and carob bean gums in these cheeses and has supported the changes made in technique by collaborative study. The matter may be considered closed, as far as these natural gums are concerned. There is an official method for the detection of gelatin in cottage cheese.[3] The only work yet to be done is the development of a method for the detection of algin in cheese. Your Referee concurs in the Associate Referee's recommendations.

The Associate Referee on gums in cacao products has submitted a progress report showing partial success in the detection of stabilizers in chocolate milk. She has been able to detect natural gums such as carob bean gum, gum arabic, and Irish moss, but has had difficulty with algin. The Associate Referee made no specific recommendation, but recommended that work be continued.

No reports were received from Associate Referees on gums in catsup and related products and on frozen desserts. The Associate Referee on starchy salad dressings resigned late in the year. The subject was reassigned too late for the new appointee to do any work.

RECOMMENDATIONS*

It is recommended—

(1) That the method for detection of gums in soft curd cheeses, as modified by the Referee, be adopted as official.

(2) That work be done by the Associate Referee on gums in cheese on the detection of algin.

(3) That work be continued on the development of methods for detection of gums in cacao products.

(4) That work be continued on the detection of gums in catsup and related products.

(5) That the subject detection of gums in frozen desserts, be reassigned.

(6) That work be continued on detection of gums in starch salad dressings.

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[1] Fed. Register, 7, 10758 (Dec. 23, 1942).
[2] *Methods of Analysis*, 7th Ed. 15.139–15.142.
[3] *Ibid.*, 15.143.
* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 50 (1951).

REPORT ON ALGIN AND GUMS IN CACAO PRODUCTS

By FLORA Y. MENDELSOHN (Food and Drug Administration, Federal Security Agency, Los Angeles, Calif.), *Associate Referee*

Since the progress report of 1949, work has been continued on the detection of algin and gums in chocolate milk.

The work with gums has not presented any special problems. Samples of chocolate milk containing Irish moss, locust bean gum, and gum arabic were used. These samples were treated in the conventional manner **(15.140–15.142).** A 20% trichloracetic acid solution was used to precipitate the milk and other proteins (50 ml. freshly prepared trichloracetic to 100 ml chocolate milk). After centrifuging and decanting off the clear liquid, the gum was precipitated from the decanted liquid by the addition of 4 vol. of alcohol (95%). After washing alcohol precipitate, etc., the presence of gum was confirmed by the copper reduction test using Benedict's solution **(15.141).** The presence of 0.1% sodium benzoate in the reconstituted milk (powdered skim milk plus water) caused no interference in the precipitation of the gums.

The detection of algin in chocolate milk continues to present a problem. When a sample containing a mixture of algin and gum was tested, one portion being treated for the detection of gum and a second portion being treated with tannic acid for the separation of sodium alginate, a positive test for gum was obtained, but not for algin.

A check was made to determine if the formaldehyde in any way reacted with the algin. It was found, when 1 ml of 37% formaldehyde was added to 300 ml of 0.16% algin solution (per cent of algin in chocolate milk), no visible change took place. After filtering 25 ml of this solution a positive ferric chloride test for algin was obtained.

In view of the fact that some of the samples containing only algin gave a positive ferric chloride test while others prepared in the same manner did not, further work will be continued on the detection of algin, with special emphasis on the preparation of the sample in order to disperse the algin throughout the mixture.

REPORT ON GUMS IN SOFT CURD CHEESE

By M. J. GNAGY (Food and Drug Administration, Federal Security Agency, Los Angeles, California), *Associate Referee*

The work of the Associate Referee in 1949 was carried out with a twofold purpose: (1) to revise the present method in preparation for the contemplated publication of the seventh edition of *Methods of Analysis, A.O.A.C.* in 1950, and (2) to submit to collaborative study the revised method when used to detect gum tragacanth and gum karaya as well as locust seed (carob bean) gum in creamed soft curd cheese.

Some of the collaborators in 1949 experienced difficulty in obtaining blanks on authentic control cheese without added gum, while some had difficulty in detecting the presence of gum karaya. Consequently, no report was made and in 1950 another set of collaborative samples was sent out with a method modified in the light of the 1949 results.

1949 EXPERIMENTAL WORK

Previous work (*This Journal*, **25**, 722, and **28**, 245) showed that as little as 0.05% of locust seed gum, a so-called soluble gum, could be detected by the present tentative method and its predecessor. These two methods differ only in manipulative detail, not in principle. Hart (*This Journal*, **20**, 531, and **23**, 597) showed the former tentative method could be applied to tragacanth and karaya gums, which are so-called insoluble gums. This has been confirmed by work done by the Associate Referee. However, no collaborative work has been done under the present tentative method.

The Associate Referee has been able to detect easily the presence of 0.1% of tragacanth and karaya gums in uncreamed soft curd cheese, where the lactose and other soluble material found in milk have been well removed from the curd through washing. His work on creamed soft curd cheese showed that the presence of 0.2% and 0.15% of either tragacanth or karaya gums could be detected very easily. The Associate Referee therefore decided to submit for collaborative study samples of creamed soft curd cheese containing still smaller amounts of these two gums in order to test further the sensitivity of the method.

DESCRIPTION OF COLLABORATIVE SAMPLES

A fine-grained cottage cheese, free from gum, was secured from a local creamery and 70-gram portions were weighed into small glass jars. These jars were separated into four sample groups. 0.1 gram of tragacanth gum was added to each jar of sample No. 1; 0.1 gram of karaya gum was added to each jar of sample No. 2; no gum was added to the jars of sample No. 3; and 0.1 gram of locust seed gum was added to each jar of sample No. 4. The gum was weighed out on an analytical balance, placed in a 25-ml beaker, five ml of water added, mixture stirred, and the gums allowed to dissolve or gel. Then the portions were transferred to proper sample bottles and mixed, using ca 5 ml more of water. A mixture of milk (3.5% butterfat) and of cream (30.5% butterfat) was taken of such proportions that when 20 ml of the mixture was added to each bottle of the four samples the per cent of butterfat in the resulting creamed cottage cheese was about 4.5%. The total weight of the contents of each jar was 100 g, thereby making the gum content 0.1%.

One jar from each of the four samples was sent to each collaborator, but the collaborator neither knew which jar contained the gum nor the

TABLE 1.—*1949 collaborative results on determination of presence of various gums in creamed cottage cheese*

COLLABORATORS	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
	0.1% GUM TRAG. ADDED		0.1% GUM KARAYA ADDED		NO GUM ADDED		0.1% LOCUST SEED GUM ADDED	
	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS
B. H. Gnagy Dept. of Health Los Angeles, Calif.	Large amt. of red precipitate	Present	Small amt. of red precipitate	Present	No analysis made	—	No analysis made	—
J. E. Roe Food & Drug Adm. Denver, Colorado	Large in amt., yellow-orange in color	Present	Small in amt., yellow-orange in color	Present	Small in amt., light tan in color	Absent	Fairly large in amt., yellow-orange in color	Present
Sam D. Fine Food & Drug Adm. Cincinnati, Ohio	Voluminous red precipitate	Present	Slight red precipitate	Trace present	Slight red precipitate	Trace present	Voluminous red precipitate	Present
Marvin Cook June Dairy Prod. Co., Inc. Jersey City, N.J.	Fairly heavy orange-red precipitate	Present	(No comment)	Not detected	(No comment)	Not detected	Brick-red precipitate, not as heavy as No. 1	Present
David Menschenfreund Food & Drug Adm. Los Angeles, Calif.	Heavy orange precipitate	Present	Heavy orange precipitate	Present	Small orange precipitate	Probably absent	Fairly heavy orange precipitate	Present
C. E. Hynds New York State Food Lab. Albany, N.Y.	Orange red	Present	Small amount yellow-orange	Absent*	Very slight, yellow	Absent	Red	Present
Albert L. Weber Food & Drug Adm. New York, N.Y.	Heavy, red in color	Present	Heavy, dark orange in color	Present	Very small amt., light yellow in color	Doubtful, probably absent	Heavy, bright orange in color	Present
M. J. Gnagy Associate Referee	Heavy orange-red precipitate	Present	Smaller orange-red precipitate	Present	Small, flocculent, almost colorless precipitate	Absent	Heavy, orange-red precipitate	Present

* Collaborator comments: "Sample No. 2 gave some precipitate, perhaps due to very small amount of gum or some interfering substance, but would be considered negative for law enforcement."

quantity of gum. In addition, another jar of cheese was sent to each collaborator, marked "no gum added," to be used as a trial sample.

METHOD

The methods sent to collaborators in 1949 and 1950 were alike in principle. The 1950 method is included in this report together with modifications in technic from the method submitted to collaborators in 1949.

COLLABORATIVE RESULTS

The results obtained by the collaborators are shown in Table 1.

COMMENTS BY COLLABORATORS

J. E. Roe, B. H. Gnagy, and Sam D. Fine claimed to have had no particular difficulty with the method.

Fine proposed a short-cut by weighing the sample directly into the 250 ml centrifuge bottle and making fat extraction directly therefrom. This procedure was included in the method sent out to the collaborators in 1950 work.

Marvin Cook and David Menschenfreund had trouble in removing fat from the cheese.

C. E. Hynds suggested use of 25 ml beakers instead of test tubes for Benedict's test. This suggestion was adopted for the method used this year.

A. L. Weber had difficulty in centrifuging down completely the trichloracetic acid precipitate and in decanting off the petroleum ether from the water solution.

DISCUSSION OF RESULTS

In general, very good results were obtained by the seven collaborators. Trouble was encountered by some analysts in detecting 0.1% karaya gum. The precipitate obtained in the Benedict's test on the karaya gum sample was much smaller than the precipitate obtained in the samples containing gum tragacanth or locust seed gum. This may be due to loss of the gum during centrifuging operations. No difficulty was encountered by any collaborator in detecting 0.1% gum tragacanth and 0.1% locust seed gum. 0.05% gum tragacanth could have been detected. 0.05% locust seed gum has been detected.

Some difficulty was encountered by some analysts in securing a larger blank than necessary on the cheese without added gum. There was a difference of opinion as to the interpretation of such small blanks.

1950 EXPERIMENTAL WORK

Collaborative work this year was carried out in an attempt to solve the difficulties that some collaborators had in the 1949 work. The method sent out was essentially the same as sent out in 1949 with the following principal changes: (1) Introducing a second precipitation with trichloracetic acid to eliminate protein that sometimes carries by the first trichloracetic acid precipitation; (2) Centrifuging at 1200 r.p.m. when the gum is to remain in the supernatant liquid and 1800 r.p.m. when the gum is

to be precipitated out; and (3) the weighing of the sample directly into the bottle. The suggested method follows:

METHOD

REAGENTS

(*a*) *Benedict's soln (qualitative).*—Dissolve 17.3 g of Na citrate and 10 g of anhydrous Na_2CO_3 in ca 80 ml of hot H_2O; dissolve 1.73 g of $CuSO_4 \cdot 5H_2O$ in 10 ml of H_2O. Filter the alkaline citrate soln, add the $CuSO_4$ soln slowly, with constant stirring, and dilute with H_2O to 100 ml.

(*b*) *Sodium hydroxide soln.*—10%.

(*c*) *Trichloracetic acid soln.*—50%. Use fresh soln made up just before use from non-hydrolyzed reagent.

(*d*) *Dilute trichloracetic acid soln.*—10%. Note warning under (c).

(*e*) *Ammonium hydroxide.*—Reagent, 28–29% NH_3.

(*f*) *Acetic acid.*—Reagent, glacial, 99.5%.

(*g*) *Alcohol.*—95%.

(*h*) *Alcohol.*—70%.

(*i*) *Potassium aluminum sulfate soln.*—5%.

TREATMENT OF SAMPLE

Weigh into 250 ml centrifuge bottle 100 g of cheese. Add hot H_2O to make volume 170 ml. Heat in hot H_2O bath for 30 min. Cool to room temperature, add 50 ml petr. ether, shake, and centrifuge. Remove petr. ether layer by decantation or by use of a blow-off siphon. Repeat extraction with petr. ether at least twice. Small amount of fat remaining will do no harm. Warm bottle in hot H_2O bath to remove residual petr. ether from cheese. Centrifuge, if necessary, to break any foaming that may occur. Make volume to ca 190 ml with H_2O and add 3.5 ml NH_4OH a few drops at a time while stirring contents of bottle. Keep in hot H_2O bath and stir until all curd has dissolved. If curd fails to completely dissolve, add few more drops of NH_4OH, stir and macerate to secure soln. Add acetic acid, a few drops at a time, with shaking, until *p*H is ca 4.75 (nitrazine test paper or *p*H meter suitable). Use care in approaching *p*H point because isoelectric point for casein is ca *p*H 4.73. (If acid is added very slowly with constant shaking and the centrifuge bottle is kept hot, marked separation of casein and liquid will be noted at this point.) Stopper bottle, shake thoroly and allow to stand overnight in the hot H_2O bath as the H_2O cools. Check *p*H, and centrifuge at 1200 r.p.m. for 10 min. Decant supernatant liquid into 250 ml beaker with 40 ml graduation mark. Do not wash precipitate.

SEPARATION OF GUM

Evaporate decanted liquid on steam bath to 40 ml mark of beaker. Remove beaker from bath and cool to room temperature. Disregard precipitate formed during concentration and add 10 ml of 50% trichloracetic acid soln (note warning under Reagents). Replace on steam bath for at least 15 min to coagulate protein. Remove beaker from steam bath, cool, transfer to 250 ml centrifuge bottle with 5 ml of the dilute trichloracetic acid soln and centrifuge at 1200 r.p.m. for 10 min. Decant supernatant liquid into another 250 ml centrifuge bottle and add alcohol with stirring until bottle is full. Allow mixture to stand at least 1 hr to coagulate gums. Centrifuge at 1800 r.p.m., decant and discard liquid. (The volume before addition of alcohol should not exceed 50 ml and ca 4 volumes of alcohol should be added.)

TABLE 2.—*1950 Collaborative results on determination of presence of various gums in creamed cottage cheese*

COLLABORATORS	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE KNOWN TO CONTAIN NO ADDED GUM	
	0.1% GUM TRAG. ADDED		NO GUM ADDED		0.2% GUM KARAYA ADDED			
	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS	BENEDICT'S TEST	GUMS
B. H. Gnagy, Dept. of Health Los Angeles, Calif.	Much red precipitate	Present	No precipitate	Absent	Small amount of red precipitate	Present	No precipitate	Absent
J. E. Roe, Food & Drug Adm. Denver, Colo.	Large amt. of red orange precipitate	Present	No precipitate	Absent	Small amount of red orange precipitate	Present	No precipitate obtained	Absent
C. E. Hynds, New York State Food Lab., Albany, N.Y.	Orange precipitate	Present	No precipitate	Absent	Orange red precipitate	Present	No precipitate	Absent
Albert L. Weber, Food & Drug Adm. New York, N.Y.	Heavy red precipitate	Present	Light precipitate, yellow in color	Present (trace)	Light precipitate, yellow orange color	Present	No precipitate	Absent
M. J. Gnagy, Associate Referee	Very large red precipitate	Present	No precipitate	Absent	Smaller orange red precipitate	Present	No precipitate	Absent

Add to residue in bottle ca 50 ml of 70% alcohol, stopper and shake to thoroly break up material. Wash down stopper and sides of bottle with a little 70% alcohol, centrifuge at 1800 r.p.m., decant and drain. Add 40 ml hot H_2O to bottle and shake well to dissolve gum and disperse insoluble material. Add 10 ml of 50% trichloracetic acid to bottle and heat on steam bath for 15 min to coagulate any protein left after first treatment. Remove bottle, cool and centrifuge at 1200 r.p.m. for 10 min. Decant supernatant liquid into another 250 ml pyrex centrifuge bottle. Fill bottle with 95% alcohol while stirring contents. Add 0.5 ml of 5% potassium aluminum sulfate soln (Reagent i). Shake and allow to stand at least 1 hr. Centrifuge at 1800 r.p.m. and decant. Add 50 ml of 70% alcohol, shake to disperse material and centrifuge at 1800 r.p.m. Decant off supernatant liquid and drain. Add 40 ml of hot H_2O and shake well to dissolve gum. Transfer to 50 ml capacity conical heavy duty centrifuge tube, keeping volume to 40 ml. Centrifuge at 1200 r.p.m. for 10 min to remove any undissolved material and decant supernatant liquid back into 250 ml centrifuge bottle. Reprecipitate in bottle by filling with alcohol plus 1 drop of acetic acid. To insure precipitation of gum tragacanth and karaya, add 0.5 ml of potassium aluminum sulfate soln.

DETECTION OF GUM

Allow precipitate to coagulate as before. Centrifuge and decant off liquid. If the precipitate is small in amount and will not remain on bottom of 250 ml centrifuge bottle, centrifuge, a portion at a time, the alcohol and precipitated gum at 1500 r.p.m. for 15 min in a 50 ml capacity conical heavy duty centrifuge tube until all contents of 250 ml bottle have been transferred to 50 ml tube. After decanting supernatant liquid from last portion centrifuged, add 40 ml of 70% alcohol to tube (or bottle if tube is not used), shake until precipitate is dispersed, centrifuge, decant, and drain.

Add to residue in tube or bottle 10 ml of hot H_2O, shake and transfer contents to 50 ml beaker. Rinse out tube or bottle with 10 ml of hot H_2O and add H_2O to beaker. Warm on electric hot plate to dissolve gum and evaporate to 10 ml. Add 2 ml conc HCl, cover beaker with watch glass and boil gently 5 min. Cool and transfer to 10 ml graduated cylinder. Adjust to 10 ml with H_2O and mix. Place 1 ml aliquot in 30 ml beaker and neutralize with NaOH soln using litmus paper as indicator. Remove litmus paper, add 5 ml of Benedict's soln and boil vigorously 2 min. Allow contents of beaker to cool spontaneously. Voluminous precipitate appearing on cooling, which may be yellow, orange or red, caused by reducing sugars formed by hydrolysis of the gums, indicates presence of gums.

RESULTS

The results obtained by the collaborators are shown in Table 2.

COMMENTS BY COLLABORATORS

B. H. Gnagy.—Suggest that the use of potassium aluminum sulfate be mentioned earlier.

J. E. Roe.—No particular difficulties were encountered. The present method of removing fat is much better than that used in 1949. The method is a little longer but the results are certainly worth the extra time involved.

C. E. Hynds.—No difficulty encountered. The method seems to work very well.

Albert L. Weber.—The cheese had a tendency to froth over while bringing the *p*H to 4.73 with acetic acid. This year I had difficulty in determining the proper end point with nitrazine paper.

DISCUSSION OF RESULTS

Three of the collaborators secured the same results as the Associate Referee did upon the four samples sent out. The fourth collaborator secured the same results on three samples but secured a slight precipitate with Benedict's solution on sample 2 where no gum was added. In his comment he mentions the trouble he had in securing the proper *p*H with nitrazine paper. He evidently failed to secure the optimum condition for the precipitation of protein on sample 2 and hence sufficient reducing substances were carried by to give the slight precipitate with the Benedict's solution.

CONCLUSION

The standards for cream cheese and Neufchatel cheese provide for the addition of 0.5% of gum tragacanth, gum karaya, or locust seed (carob bean) gum, or a mixture containing two or more of these gums. Judging from the results of the collaborative work of the last two years and from results obtained by the Associate Referee in other years, it would appear that the method submitted this year (a revision of the present first action method) has a sensitivity of approximately 0.05% for gum tragacanth and locust seed (carob bean) gum and a sensitivity of approximately 0.1% for gum karaya. This sensitivity is well within the standard that has been set.

RECOMMENDATIONS*

It is recommended—

(1) That the method given herein, which differs only in manipulative details from that of **22.138–22.141** (Methods, 1945) which was made first action in 1949, now be made official.

(2) That collaborative study be undertaken on the detection of alginates in soft curd cheese.

REPORT ON VITAMINS

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

At our meeting last year we heard reports from Associate Referees on vitamins that reviewed the status of all vitamin methods previously adopted, with recommendations that brought them into accord with the recently accepted method classification. A number of these methods are official and no further study of them has been conducted during the past year. For this reason there are fewer reports on our program this year.

* For report of Subcommittee C and action of the Association, see *This Journal*, 34, 50 (1951).

I am informed that the Associate Referee on Vitamin B_{12} has made certain preliminary studies that he does not regard as sufficient basis for a report this year. On the basis of these and of the collaborative work sponsored by the U. S. Pharmacopoeia, he plans to proceed during the coming year with study of a method designed for determination of the vitamin B_{12} content of foods and animal feeds.

The Associate Referee on Vitamin A in Mixed Feeds has continued that work in accordance with Dr. Schaefer's recommendation of last year and has presented the results of the collaborative work. I concur in the recommendation* of Dr. Cooley, which is as follows.

"According to the consensus of the collaborators the Referee recommends the method for adoption, first action, subject to further collaborative study."

Although no further work has been done on the method for vitamin D in poultry feeds, further experience justifies the recommendation* that the method be made official.

It has been announced that the new U.S.P. Vitamin D Reference Standard has now become available, though the date that it will be made official has not been given. I call to your attention the recommendation of the Associate Referee, made last year in this connection that when the new U.S.P. standard becomes official: "(1) the term *U.S.P. Reference Cod Liver Oil* be deleted wherever it appears in the method and the term *U.S.P. Vitamin D Reference Standard* replace it, (2) that the term *A.O.A.C. Chick Unit* be deleted wherever it appears in the method and the term *International Chick Unit* replace it." This will take effect on the date the new U.S.P. standard becomes official.

It is recommended* that the microbiological method for folic acid adopted first action last year, now be made official.

A new Associate Referee on a chemical method for nicotinic acid was appointed this year and Dr. Sweeney's report of his collaborative work has been presented. I concur in his recommendation* that the chemical method for the determination of nicotinic acid by the sulfanilic acid procedure with phosphate buffer modification be adopted, first action.

Dr. Quackenbush has continued to work on the determination of carotene in accordance with his recommendations of last year. His report indicates the progress that has been made. I concur in his recommendation* which was as follows:

"It is recommended that studies on carotene analysis be continued."

* For report of Subcommittee A and action of the Association, see *This Journal*, 34, 44 (1951).

REPORT ON VITAMIN A IN MIXED FEEDS

By MAXWELL L. COOLEY (General Mills, Inc., Minneapolis, Minnesota), *Associate Referee*

Because of its high nutritional importance and the prevalent use of true vitamin A in mixed feeds there has been an obvious and growing necessity for a satisfactory chemical procedure for the measurement of this vitamin in these products. Therefore, in 1949 a collaborative study as a means of establishing such a method for the A.O.A.C. was instigated by Dr. H. C. Schaefer (6). The report of this work was of great interest and value. The data and information contained therein served as a good indication that a practical and acceptable procedure could be developed.

Dr. Schaefer brought out the fact that several methods applying to the measurement of vitamin A in mixed feeds have been published. These were listed chronologically to include the methods of Cooley, Christiansen and Schroeder (2), Brew and Scott (1), Narod and Verhagen (5), Wall and Kelley (7), and a revised method by Cooley, Christiansen, and Koehn (3). Perusal of these papers is recommended.

A questionnaire as well as the tentative revamped procedure and remarks concerning the proposed procedure were mailed early this year to 35 laboratories which had indicated a wish to partcipate in the A.O.A.C. study.

Detailed study of last year's report, the remarks, comments and criticisms of the collaborators who previously participated, and the answers to the current questionnaires revealed considerable information and ideas. With this in mind a final draft was drawn up of the procedure to be used for the assay of Vitamin A. This is given below.

The following method was sent to all collaborators to be used on the samples submitted to them:

VITAMIN A IN MIXED FEEDS

APPARATUS

(1) *Photoelectric colorimeter.*—An Evelyn Colorimeter equipped with a 620 millimicron glass filter or a Coleman Spectrophotometer has been found to be desirable. An instrument with direct reading, deflecting type galvanometer is necessary.

(2) *Chromatographic tubes.*—Cylinder 23×200 mm, sealed to 5×80 mm tube. (Wilkens-Anderson Co., Chicago, Ill.)

(3) *Graduated cylinder.*—100 ml lipless, or cut off top of a regular 100 ml graduated cylinder just below lip and fit with a suitable 2-hole rubber stopper (or use any other desirable receiver for eluate from adsorption column).

(4) *Fat extraction apparatus.*—Goldfisch, Bailey-Walker, or Soxhlet.

(5) *Automatic pipette.*—10 ml. This pipette should deliver rapidly thru an opening 3 to 4 mm in diameter. (A suitable size graduated cylinder may also be used effectively for dispensing Carr-Price reagent.)

REAGENTS

(1) *Hexane.*—B. P. 60–71 degrees C. (Skellysolve B).

(2) *Acetone.*—Reagent grade.

(3) *10% Acetone in hexane.*—(Skellysolve B).

(4) *Absorbent.*—Equal parts by weight of diatomaceous earth (Johns-Manville Hyflo Super Cel) and Magnesia (Micron brand #2641-Westvaco Chlorine Products Corporation, Newark, California).

(5) *Anhydrous sodium sulfate.*—Reagent grade.

(6) *Chloroform.*—Reagent grade (Purify by distillation if necessary to prevent color interference).

(7) *Antimony trichloride reagent.*—(Carr-Price). Prepare by dissolving 20 g of antimony trichloride in sufficient chloroform to make 100 ml. Add 3 ml acetic anhydride. Filter if necessary. (See Note 1.)

PREPARATION OF ADSORPTION COLUMN

Pass the stem of the chromatographic tube thru a two-hole stopper of proper size to fit in the top of the lipless 100-ml graduated cylinder (eluate receiver). Thru the other hole in the stopper insert a bent glass tube and connect this to a source of vacuum. Ordinarily, an efficient water pump will be sufficient. If another type of chromatographic assembly is employed an eluate receiver other than the lipless graduated cylinder may be used.

Place a *small* amount of cotton at the bottom of the chromatographic tube and pack tightly to a depth of 100 mm with a well-blended mixture of equal parts by weight of Hyflo Super Cel and magnesia. In order to assure a homogeneous blend of these two components, mix by rubbing out lumps by hand or run the mixture thru a kitchen flour sifter and roll on paper at least 50 times. Add the mixture in several portions, tamping well with a stopper or similar device. Keep suction on the column during packing. Add a 1 cm layer of anhydrous sodium sulfate to the top of the column. Do not wet column before passage of soln thru the column.

PROCEDURE

Extraction.—Weigh 10 g of feed directly into fat extraction flask. Weigh ca 1 g of dehydrated alfalfa meal into the same flask. (This serves as a source of carotene which facilitates locating by visual examination the carotene band in the chromatogram.) Add exactly 100 ml hexane and reflux on fat extractor for 30 min. Mark flask at 100 ml level with fine pointed china marking pencil. Remove flask from extractor, cover and cool for 10–15 min. Check for loss of solvent and add more if necessary to bring up to 100 ml level, mix, and allow to settle. Volume of soln may be checked also by weighing flask and contents before and after extraction.

Chromatography.—Draw a 50-ml aliquot of the supernatant liquid thru the adsorption column with suction. Elute with ca 35–40 ml of 10% acetone in hexane, using only enough of this reagent so that the first portion of the carotene band passes thru the column. A trace of carotene is eluted from the column at this point to insure complete elution of vitamin A. The eluate should, therefore, possess a distinct yellow color. Disconnect receiver from bottom of adsorption column, make up to a suitable volume, which is usually 50 ml, and mix.

Colorimetry.—Evaporate with mild heat and reduced pressure a suitable aliquot of this soln and dissolve the residue in sufficient chloroform so that 1 ml after the addition of the antimony trichloride reagent will give transmittance readings which are within the range of 30–65 per cent (absorbencies of ca 0.5–0.2). Set colorimeter at 100 per cent transmission, using a blank comprised of 1 ml of chloroform and 10 ml of Carr-Price reagent. Place the assay tube in the colorimeter and add rapidly

(automatic pipette) 10 ml of Carr-Price reagent, using 620 mμ wave length light. Take the maximum colorimetric reading (color begins to fade within 3–5 seconds). Determine units of vitamin A in tube from standard curve and calculate units of vitamin A per gram of feed. (See Note 2.)

NOTE 1.—A few precautions are necessary in the preparation of the antimony trichloride in chloroform soln. The use of a fresh bottle of antimony trichloride crystals which has not been opened previously is desirable. Even then for best results the crystals should possess a translucent appearance. Crystals which have been stored too long or are kept in a bottle which has been opened, frequently contain an objectionable brown-colored decomposition material. The antimony trichloride plus the prescribed volume of chloroform are merely heated on a hot plate until solution takes place. The soln is cooled and 3 per cent of acetic anhydride is mixed in. The reagent thus prepared usually is clear; if not it may be easily centrifuged, filtered or allowed to settle and decanted. It may be stored in a brown bottle for several months.

Contact with water causes the formation of the heavy white precipitate, antimony oxychloride. The use of concentrated or slightly diluted hydrochloric acid is an effective and simple way of cleaning and removing this precipitate from apparatus and glassware.

NOTE 2.—The official standard to use for development of the colorimetric curve in this work is the U.S.P. Vitamin A Reference Standard obtainable from the United States Pharmacopoeia Reference Standards, 4738 Kingsessing Avenue, Philadelphia 43, Pa. This is in the form of capsules each of which contain close to 2500 units of vitamin A. The potency of the oil in these capsules is 10,000 units of vitamin A per gram. Although a curve established using the whole oil is recommended, results within the accuracy of the proposed procedure may be obtained by use of a curve based on the unsaponifiable fraction of the oil inasmuch as the two curves are very similar. In making standard solutions directly from the vitamin A standard the end of a capsule may be removed with scissors and exactly 0.2 g weighed out on a small watch glass. This is washed into a 50 ml volumetric flask with chloroform and made up to volume. Each ml of this soln contains 40 units of vitamin A. A series of dilutions in chloroform are made from this standard soln so that 1 ml aliquots when placed in individual colorimeter tubes develop intensities of color which spread over the range of the colorimeter. Suggested concentrations in consecutive tubes might be 5, 10, 20, 30 and 40 units of vitamin A. From the resulting color readings a standard colorimetric curve is established.

It has been generally agreed that the best and least difficult method of following the vitamin A through the adsorption column is to watch the carotene band as it progresses down the column and begins to be eluted. However, many feeds do not contain sufficient carotene to form a distinct and discernible band of this pigment. Any good source of carotene could be used, but no doubt good quality dehydrated alfalfa meal would be the most readily available and desirable carotene-bearing material found in the average feed testing laboratory. The addition of anywhere from 0.5 to 2.0 grams of dehydrated alfalfa meal to the extraction flask after the sample has been placed in the flask and before the solvent has been added should furnish sufficient carotene to form a very distinct band in the chromatogram. Some of this carotene band must

pass out of the column to insure complete elution of the vitamin A band. The fact that carotene reacts with antimony trichloride in chloroform more slowly than does true vitamin A to form a maximum color, plus the fact that the resulting carotene blue color displays a maximum light absorption at a somewhat lower wave length than does the Carr-Price vitamin A color, tends to mitigate the effect of the presence of some carotene in the final eluted vitamin A solution. Obviously the per cent error caused by carotene being read spuriously as vitamin A is greatest when the vitamin A potency of the feed being assayed is low.

In view of the fact that the method under study necessarily omits saponification, consideration must be given the effect which lipids and other extracted materials also have on the color reaction. The method does not determine vitamin A alcohol as this form of the vitamin is preceded by carotene in passing through the adsorption column. Inasmuch as probably 95 per cent or more of the vitamin A in fish oils is in the ester form (4), and the ester is eluted before the carotene, the maximum error expressly from this source should be approximately 5 per cent. Inasmuch as the antimony trichloride in chloroform reagent is not specific for vitamin A and presence of some interfering materials is to be expected, the questionnaire which was sent to the collaborators asked whether the method should be designated as a determination or an estimation of vitamin A in mixed feeds. Of the questionnaires returned, over 75 per cent expressed the opinion that the procedure be termed an estimation.

Although activated glycerol dichlorhydrin also lacks specificity as a reagent for vitamin A, it has several distinct advantages. It is not corrosive like the antimony trichloride solution, is easily washed off, and may be purchased ready for use. The color reaction with vitamin A is more stable than the Carr-Price blue color.

However, the Carr-Price reaction is considerably more sensitive and is the most universally used. In addition the A.O.A.C. and the U.S.P. have now adopted this as an official identification test for vitamin A. Therefore, it was deemed advisable to continue the use of this color reaction in the proposed method.

The desirability of the use of an internal vitamin A standard is questionable from the standpoint of accuracy and because of the difficulty in keeping a reliable vitamin A standard solution on hand due to its instability.

The use of 30 per cent acetone in hexane as extracting solvent has been deleted from the previous proposed procedure. The recommended use of plain hexane (Skellysolve B) for this purpose eliminates the required evaporation of the acetone solution prior to chromatographing. Numerous collaborators believed that a shorter extraction period is desirable, therefore the recommended time of extraction has been reduced to 30 minutes.

DISCUSSION OF COLLABORATIVE SAMPLES

Six mixed feed samples were submitted to each collaborator. Each sample contained a certain added amount of true vitamin A. Samples 1 through 5 derived their vitamin A potencies from added fish liver oil and Sample 6, from an added dry vitamin A preparation. The composition of the experimental feed mixtures is given in Table 1.

TABLE 1.—*Composition of experimental feed mixtures*

SAMPLE	1	2	3	4	5	6
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wheat bran	10	15	5	—	—	—
Wheat middlings	15	10	5	15	15	15
Corn gluten meal	10	—	5	—	—	—
Ground corn	—	20	50	15	50	50
Oatmeal	—	—	—	15	—	—
Soybean oil meal	30	35	20	15	15	15
Dehydrated alfalfa	15	10	5	—	15	15
Meat scraps	10	10	5	—	—	—
Dried skimmilk	—	—	—	20	—	—
Dried buttermilk	—	—	—	20	—	—
Fishmeal (vitamin A-free	10	—	5	—	5	5
Vitamin A (calculated units A per g.)	—	—	17	11.5	—	—

The calculated vitamin A potencies of Samples 3 and 4 are given. The calculated vitamin A values for Samples 1, 2, 5, and 6, were not divulged.

As is apparent from the composition of Sample 4, this feed is practically devoid of carotene. When assaying a mixed feed such as this or even feeds containing a small amount of carotene, the use of a carotene source is essential to provide sufficient quantities of this pigment in the extracted solution to permit following visually the band in the chromatogram. A sample of dehydrated alfalfa meal was also sent to each collaborator for this purpose.

In order to examine the proficiency of each laboratory as to precision of their calibration curves and color measurement and the reproducibility of results by the Carr-Price reaction only, excluding the extraction and chromatographic procedure, a portion of the vitamin A oil which was used in preparing feed mixtures 1 through 5 was included in the collaborative samples.

The procedure to be used in assaying this vitamin A oil was described. The procedure omits saponification and specifies determining the vitamin A in the whole oil as follows:

Procedure for Vitamin A in A.O.A.C. Collaborative Vitamin Oil Sample

Weigh 0.15–0.20 g[1] of whole oil on small watch glass. Rinse into a 100 ml volumetric flask with chloroform, make up to volume with chloroform, and mix. Place 1 ml of this soln in a colorimeter tube. Set colorimeter at 100 per cent transmission, using a blank comprised of 1 ml of chloroform and 10 ml of Carr-Price reagent. Place the assay tube in the colorimeter and add rapidly (automatic pipette) 10 ml of Carr-Price reagent. Using 620 mμ light, take maximum colorimetric reading (color begins to fade within 3–5 seconds). Determine units of vitamin A in tube from standard curve and calculate units of vitamin A per g of oil.

LIST OF COLLABORATORS

R. E. Anderson, Archer Daniels Midland Co., Minneapolis, Minn.
F. A. Bacher, Merck and Company, Inc., Rahway, N. J.
B. H. Barrows and D. H. Leweke, Hales and Hunter Co., Chicago, Ill.
M. L. Cooley* and J. J. Hoffman, General Mills, Inc., Rossford, Ohio.
W. R. Flach and C. D. Sander, Eastern States Farmers Exchange, Buffalo, N. Y.
P. R. Frey, Colorado A. and M. College, Fort Collins, Colo.
F. H. Hedger, Chas. Pfizer and Co., Brooklyn, N. Y.
H. H. Hoffman, Florida Department of Agriculture, Tallahassee, Fla.
C. H. Krieger and Ruth Nell, Wisconsin Alumni Research Foundation, Madison, Wis.
J. W. Kuzmeski and A. F. Spelman, Agr. Exp. Sta., Massachusetts State College, Amherst, Mass.
R. L. Matthews, M.F.A. Milling Co., Springfield, Mo.
D. J. Mitchell, South Dakota State Chemical Laboratory, Vermillion, So. Dak.
K. Morgareidge, Nopco Chemical Co., Harrison, N. J.
M. Narod, Lyle Branchflower Co., Seattle, Wash.
B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.
D. B. Parrish, Kansas State College, Dept. of Chemistry, Manhattan, Kan.
F. E. Randall, Cooperative G.L.F. Exchange, Buffalo, N. Y.
E. D. Schall and F. W. Quackenbusch, Agr. Exp. Sta., Purdue University, Lafayette, Ind.
H. C. Schaefer, W. B. Brew, and A. Schulz, Ralston Purina Co., St. Louis, Mo.
W. S. Thompson and D. M. Stalter, State of Ohio Dept. of Agr., Columbus, Ohio.
J. B. Wilkie, Food and Drug Administration, Washington, D. C.
R. E. Zenk, H. J. Heinz Company, Pittsburgh, Pa.

COLLABORATIVE DATA

Table 2 presents the average values reported by twenty-two collaborators for the six feed samples and the one vitamin A oil sample using the proposed chromatographic procedure and the specified Carr-Price procedure as applied to whole oil, respectively. The majority of the values given in Table 2 represent the average of at least three individual analyses on each sample.

[1] With this weight of this particular oil sample and the volume of chloroform specified, a 1-ml aliquot when reacted with 10 ml of Carr-Price reagent should give transmission readings which are within the range of 30 to 65 per cent (absorbencies of approximately 0.5 to 0.2).

* Present address: General Mills, Inc., Minneapolis, Minn.

TABLE 2.—*Average values found for vitamin A in collaborative samples*

COLLABORATOR	SAMPLES (UNITS OF VITAMIN A PER GRAM)						
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	VIT. A OIL
1	6.5	10.2	15.7	11.3	15.2	13.4	11,200
2	8.9	13.2	20.8	11.0	20.2	16.8	14,000
3	6.2	8.9	16.0	10.4	15.0	14.2	11,400
4	7.4	10.1	14.8	9.7	15.3	15.7	11,200
5	6.1	9.3	16.7	9.1	15.9	16.3	11,600
6	8.4	12.0	17.6	11.6	17.6	16.0	13,100
7	8.3	11.1	17.3	11.1	17.1	14.6	13,100
8	12.0	11.7	16.9	11.5	20.5	16.1	10,700
9	6.9	9.9	15.5	10.4	17.0	16.0	11,500
10	4.8	5.7	8.9	5.5	8.5	9.0	11,400
11	15.9	14.8	21.3	15.2	24.9	23.8	12,800
12	6.5	9.1	14.9	9.7	14.8	15.0	11,900
13	7.7	10.2	17.1	9.3	18.1	16.9	14,500
14	8.6	10.8	16.9	11.8	18.8	15.7	12,000
15	7.0	10.3	16.6	10.4	16.4	14.4	12,900
16	5.9	7.8	13.6	6.2	9.3	8.8	13,600
17	8.0	10.9	17.7	10.5	18.5	17.2	12,600
18	10.0	11.0	16.8	10.3	19.5	20.3	12,300
19	7.9	10.4	17.1	10.9	16.6	18.2	12,900
20	8.3	9.7	17.3	11.7	19.3	18.5	13,300
21	17.7	12.3	17.1	11.4	22.3	20.2	12,100
22	7.3	10.0	16.0	10.7	17.7	16.1	13,100
Calculated Value	7.5	11.5	17.0	11.5	17.0	15.5	13,000*
Mean	8.47	10.43	16.48	10.44	17.20	16.05	12,418
Standard Deviation	3.03	1.95	2.21	2.10	3.59	3.48	387
Coefficient of Variation	35.7%	18.7%	13.4%	20.1%	20.8%	21.6%	3.1%

* The potency of this oil sample was determined by both the blue color method and spectrophometrical method by 2 laboratories. Average results showed this to be a 13,000 A oil.

COMMENTS AND SUGGESTIONS BY COLLABORATORS

Of the twenty-two collaborators who submitted results, eighteen had either participated in the 1949 collaborative study for vitamin A in mixed feeds or had previous experience with this type of chemical assay.

The procedure as given was clear and easily understood according to answers to the questionnaire which was sent out.

The comments and suggestions of collaborators are in part given below:

Collaborator No. 1.—Variation in the method as used by us: Elution of vitaminA is carried out until trace of carotene passes thru column, the eluate being caught in a 125-ml Erlenmeyer. Three glass beads are added to each of the flasks, which are then placed in a bed of granulated aluminum contained in a vacuum distillation apparatus (Fisher #9-137, equipped with water bath), and reduced to dryness with

heat and vacuum. Flasks are cooled under running water and 2 ml $CHCl_3$ are added to each. Residue is dissolved and a 1-ml aliquot transferred to a colorimeter tube.

Collaborator No. 4.—In general the method worked very well and gave excellent checks. However, there is some doubt about the vitamin A alcohol and in general about the fate of the missing A.

Collaborator No. 7.—We feel that your procedure is more satisfactory for feeds than any we have encountered to date . . . . For the Coleman Model 14, we have found it more convenient to take up the residue from the evaporated aliquot in 0.5 ml of chloroform and add 4.5 ml of Carr-Price reagent to produce color. In this manner we keep the volume for instrument reading at a minimum and obtain a high sensitivity and a thorough mixing of the reagent in the cuvette.

Collaborator No. 8.—Pressure instead of suction was used to force the solvent thru the chromatographic column. The volume of eluate was reduced to 25 ml. Aliquots were transferred to a colorimeter tube and evaporated to dryness under a stream of nitrogen. One ml of chloroform was added taking care to wash down the sides of the tube.

Collaborator No. 14.—We had no trouble with the procedure, and the only comment we have is that the time required for the sample and solvent to cool after extraction is considerably more than 10–15 minutes.

INTERPRETATION OF COLLABORATIVE DATA

In reviewing the results of this collaborative work consideration must be given to the possible sources of error inherent in the proposed procedure.

The blue color test for vitamin A (Carr-Price reaction) is considered not as accurate as the spectrophotometric method, especially when the Morton-Stubbs correction factor is applied to the latter. An indication of this is given by an excerpt of an article by Lawrence Rosner which appeared in the January 7, 1950, issue of *Feedstuffs*, as follows: "The new A.O.A.C. method, as presently written, is identical with that of the U.S.P. This includes calculation of vitamin A potency by multiplying the 'E value' corrected for non-vitamin A absorption by a conversion factor of 1900; also vitamin A must be determined by the blue color method ('identity test'). The ratio of the blue color value to the spectrophotometric value must be between 1.0 and 1.3. The implication is that if this ratio criterion is not met then the spectrophotometric method is not applicable and a bioassay may be required. On the basis of very few reports it appears that even with oils which have a blue color-spectrophotometric ratio in excess of 1.3 the vitamin A value based on the spectrophotometric determination is still a reliable index to biological potency."

Referring to the collaborative results obtained on the vitamin A oil sample, there are found a few fairly low results as well as a small number of rather high figures. Apparently a collaborator obtaining high results on the oil does not always indicate that the same collaborator will produce high values on the feed samples; similarly there are no definite trends in regard to collaborators who found lower potencies in the oil sample.

Collaborative data on the vitamin A oil sample further indicate that

TABLE 3.—*Average values found for vitamin A in collaborative samples excluding results which showed a variation of more than plus or minus 20 per cent from the calculated potencies*

COLLABORATOR	SAMPLES (UNITS OF VITAMIN A PER GRAM)					
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6
1	6.5	10.2	15.7	11.3	15.2	13.4
2	8.9	13.2	—	11.0	20.2	16.8
3	6.2	—	16.0	10.4	15.0	14.2
4	7.4	10.1	14.8	9.7	15.3	15.7
5	6.1	9.3	16.7	9.1	15.9	16.3
6	8.4	12.0	17.6	11.6	17.6	16.0
7	8.3	11.1	17.3	11.1	17.1	14.6
8	—	11.7	16.9	11.5	—	16.1
9	6.9	9.9	15.5	10.4	17.0	16.0
10	—	—	—	—	—	—
11	—	—	—	—	—	—
12	6.5	—	14.9	9.7	14.8	15.0
13	7.7	10.2	17.1	9.3	18.1	16.9
14	8.6	10.8	16.9	11.8	18.8	15.7
15	7.0	10.3	16.6	10.4	16.4	14.4
16	—	—	13.6	—	—	—
17	8.0	10.9	17.7	10.5	18.5	17.2
18	—	11.0	16.8	10.3	19.5	—
19	7.9	10.4	17.1	10.9	16.6	18.2
20	8.3	9.7	17.3	11.7	19.3	18.5
21	—	12.3	17.1	11.4	—	—
22	7.3	10.0	16.0	10.7	17.7	16.1
Calculated Value	7.5	11.5	17.0	11.5	17.0	15.5
Mean	7.5	10.8	16.4	10.7	17.2	15.9
Standard Deviation	0.87	0.6	1.02	0.245	1.96	0.245
Coefficient of Variation	11.6%	5.5%	6.2%	2.3%	11.3%	1.5%

there may be considerable variation from laboratory to laboratory in measurement of the blue color as well as accuracy of standard colorimetric curves.

Table 3 excludes results on the mixed feed sample which appeared to be unreasonably erratic. Examination of this table shows that 75 per cent or more of the collaborators obtained results which were within plus or minus 20 per cent of the calculated value in each sample. This indicates that the method provides an accuracy which is well within the limits of error for an estimation of vitamin A procedure. Results on sample 6, which derived its vitamin A from a "dry A" preparation, were in as close agreement as those obtained on samples prepared with fish liver oil.

SUMMARY

A proposed procedure for the estimation of vitamin A in mixed feeds was subjected to collaborative assay in 1950, and results show a definite

improvement in agreement between laboratories as compared with the previous year's collaboration. The chemical method used in the present study was a modification and simplification of the previous method. The samples submitted to the various laboratories in this work covered a somewhat wider range of vitamin A content than those of the first collaboration. In addition, there was included a whole fish liver oil sample as well as a sample of feed prepared with a dry vitamin A material. Results obtained by 75 per cent or more of the collaborators were as close to the calculated values on the samples as might be expected for this type of chemical assay. Indications are that the method should be valuable in estimating the vitamin A content of mixed feeds.

ACKNOWLEDGMENT

Appreciation and grateful acknowledgement is extended to Kenneth Morgareidge, Nopco Chemical Company, Harrison, New Jersey, for furnishing the vitamin A oil and dry vitamin A product, as well as information and data pertaining thereto which were used in preparation of the collaborative samples employed in this study.

RECOMMENDATION*

According to the concensus of the collaborators the Referee recommends that the method be adopted as first action, subject to further collaborative study.

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No report was given on pantothenic acid.

* For report of Subcommittee A and action of the Association, see *This Journal*, **34**, 44 (1951)

REPORT ON VITAMIN C IN EVAPORATED MILK

By WALLACE L. HALL (Division of Nutrition, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

A review of the literature has shown there are two adequate procedures which may be used to determine the vitamin C content of evaporated milk by use of the indophenol dye. The University of Wisconsin group[1] use photoelectric colorimeter and the Pennsylvania State College group[2] use a simple titration procedure. In both cases success was due to the use of meta phosphoric acid as a precipitant of milk proteins, and after filtration analyzing the resulting serum.

Extensive studies, carried out by the above authorities upon samples of of evaporated milk ½ to 6 months old and from all parts of the United States, led to the conclusion that for practical purposes there is no need to analyze for the oxidized form of vitamin C, 1-dehydro ascorbic acid, in evaporated milk.

REPORT ON A CHEMICAL METHOD FOR NICOTINIC ACID

By J. P. SWEENEY (Division of Nutrition, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Methods for the chemical determination of nicotinic acid have been under study in the Division of Nutrition of the Food and Drug Administration for the past year and a half.

Previous methods were based on the rupture of the pyridine ring of nicotinic acid, with cyanogen bromide, and the coupling of the resulting compound with an aromatic amine. The yellow color thus obtained was a measure of the nicotinic acid concentration. In order to extract the niacin from food and feed products and to convert niacinamide and combined niacin to free niacin, it was necessary to heat with acid or alkali. This extraction process resulted in the production of a yellow colored extract. Since it is not possible to measure accurately a yellow nicotinic acid color in the presence of a yellow colored extract, it was necessary to resort to adsorption, elution, and decolorization procedures before the development of the nicotinic acid color.

A study of the interfering colors, (1, 2) produced by the extraction process, revealed that these colors had a maximum absorption at 400

[1] Woessner, Elvehjem, and Schuette, *J. of Nutrition*, 20, 327 (1940).
[2] Josephson & Doan, Pa. *Agri. Expt. Sta. Bul. 473*, 1–22, (1945).

mμ and that the absorption became progressively less at wave lengths above 400 mμ. We believed, therefore, that an ideal coupling agent would be one which produced a color having a maximum absorption as far as possible above 400 mμ.

Of the coupling compounds tested sulfanilic acid and Tobias acid were found to be the most promising. The color produced by sulfanilic acid has a maximum absorption at 450 mμ, while that produced by Tobias acid has a maximum absorption at 500 mμ. In contrast, metol, ammonia, and aniline, coupling agents used in previously published procedures, produce colors having absorption maxima at 405, 410, and 430 mμ, respectively.

It is necessary to precipitate proteins from the test solutions in order to prevent the development of turbidity when the cyanogen bromide and aromatic amine are added. Ammonium sulfate was used for this purpose. This precipitation process also removed a great deal of color from the extract. This made the use of further adsorption or decolorization steps unnecessary.

Details of the procedures developed are given below:

PROCEDURE I—SULFANILIC ACID

REAGENTS

Ammonium hydroxide.—5 ml conc. NH_4OH diluted to 250 ml.

Hydrochloric acid.—Dilute one part conc. HCl with 5 parts H_2O.

Cyanogen bromide.—10% aqueous soln. This should be prepared under a hood. Warm 370 ml H_2O in a large flask, add 40 g CN Br; warm and shake until soln is obtained. Cool and dilute to 400 ml. Do not allow CN Br to come into contact with the skin.

Sulfanilic acid.—10% soln. Place 20 g sulfanilic acid in 170 ml of H_2O. Add conc NH_4OH 1 ml at a time until soln is obtained. Adjust to *p*H 4.5 with 1:1 HCl, using bromocresol green as an outside indicator. Make up to 200 ml. If sulfanilic acid is pure, soln should be nearly colorless.

Sodium hydroxide.—10 *N*.

Nicotinic acid.—Stock soln (keep in refrigerator). 50 mg U.S.P. Reference made up to 500 ml with 95% ethyl alcohol.

Nicotinic acid.—(Secondary standard) 2 ml stock soln diluted to 25 ml with H_2O. A small portion of the stock soln is removed each day and allowed to come to room temp. for preparation of the secondary standard.

Phosphate buffer.—17.6 g KH_2PO_4+10.24 g $Na_2HPO_4 \cdot 12H_2O$. Dissolve in H_2O and dilute to 250 ml.

PROCEDURE FOR TABLETS OR CAPSULES

Preparation of sample.—Take for analysis at least 5 tablets or capsules. Dissolve by heating in a beaker with a little water. (Tablets must be ground then heated with water.) Transfer to a volumetric flask. Pipette 10 ml aliquot into a 250 ml Erlenmeyer flask. Add 10 ml conc. HCl. Evaporate on a hot plate to about 2 ml. Cool, add water, bring to *p*H 7–9 by addition of a few pellets of NaOH or KOH. Filter, if necessary, and make up to volume. (The final dilution should contain 5–8 mmg of nicotinic acid per ml. The first dilution usually contains 50–200 mmg per ml.)

Development of Color:—Make up tubes as follows:

Blank	*Standard*	*Blank*	*Sample*
1 ml standard	1 ml standard	1 ml sample	1 ml sample
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
6.5 ml H_2O	1.5 ml H_2O	6.5 ml H_2O	1.5 ml H_2O
	5.0 ml CNBr		5.0 ml CNBr
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
1 drop conc. HCl or HBr		1 drop conc. HCl or HBr	

A blank should be run for each sample.

Measure the sample soln, NH_4OH, and water into each tube from pipettes. Then add the sulfanilic acid to the blank tube, and shake. Then add one drop of conc. HCl and again mix the contents. Place in photoelectric colorimeter and adjust to 100% transmission at 450 mμ.

Add five ml cyanogen bromide soln from a pipette to the sample tube, while the tube is swirled vigorously. Exactly 30 sec. after completing the addition of the cyanogen bromide add 2 ml sulfanilic acid from a pipette, while swirling the tube. Close tube with a rubber stopper. Place in colorimeter and read at 450 mμ. (The color reaches a maximum in about two min and remains at the peak for about the same length of time. It then starts to fade slowly.)

(The addition of reagents should take place under a hood. The pipettes for addition of cyanogen bromide and sulfanilic acid should be mounted in a ringstand and should be filled by mechanical suction since the cyanogen bromide is toxic.)

Method for enriched food products and feeds.—Weigh one ounce of material into 1-liter Erlenmeyer flask. Add 200 ml 0.25 *N* H_2SO_4. Mix, heat in an autoclave at 15 lb pressure for 30 min. Cool, adjust to *p*H 4.5 with 10 *N* NaOH, using bromocresol green as an outside indicator, filter thru a rapid filter paper into a 250-ml volumetric flask, and mix. Wash residue with sufficient H_2O to fill flask to the mark. Weigh 17 g $(NH_4)_2SO_4$ into a 50-ml volumetric flask. Pipette 40 ml filtrate into the flask, shake vigorously, dilute to mark with water, mix. Filter and mix well. Take two ml for analysis.

Prepare the standard by diluting 2 ml of the stock soln to 50 ml with H_2O. Add 40 ml of this to 17 g of $(NH_4)_2SO_4$ in a 50-ml volumetric flask and dilute to the mark with H_2O. The standard now contains 3.2 mmg per ml, and in the case of a feed with a niacin claim of 16 mg per lb, the final soln will also contain 3.2 mmg per ml. Prepare tubes as follows:

Blank	*Standard*	*Blank*	*Sample*
2.0 ml standard	2 ml standard	2 ml sample	2 ml sample
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
5.0 ml H_2O	5.0 ml CNBr	5.0 ml H_2O	5.0 ml CNBr
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
0.5 ml dil. HCl	0.5 ml dil. HCl	0.5 ml dil. HCl	0.5 ml dil. HCl

(The development of color is the same as in the case of tablets except that 0.5 ml of the HCl soln is added from a pipette immediately after the sulfanilic acid. The color, in this case, comes to a maximum at once and is less stable than in the previous case.)

(If the standard and sample are of ca same concentration, the nicotinic acid content is proportional to the photometric density.)

Modified Method of Color Development

Blank	*Standard*	*Blank*	*Sample*
2 ml standard	2 ml standard	2 ml sample	2 ml sample
2 ml phosphate buffer	2 ml phosphate buffer	2 ml phosphate buffer	2 ml phosphate buffer
5 ml H_2O	5 ml CNBr	5 ml H_2O	5 ml CNBr
2 ml sulfanilic acid	2 ml sulfanilic acid	2 ml sulfanilic acid	2 ml sulfanilic acid

PROCEDURE II—TOBIAS ACID

REAGENTS

Tobias acid (2-naphthylamine-1-sulfonic acid).

Sodium hydroxide.—10 *N*.

Ammonium hydroxide.—1 vol. concentrated ammonium to 2 vols. water.

Make up the Tobias acid soln in the following manner:

Add 10 g Tobias acid to 75 ml warm water (not over 70°C.). Add drop by drop 10 *N* NaOH until soln is obtained. Then adjust the *p*H to exactly 4.5 by the addition of 1:1 HCl. Decolorize by the addition of ca 1 g Norite and warming 5 min. Filter thru a rapid filter, make up to 100 ml, and store in a dark bottle. (This soln will become colored after standing a few days.)

(Other reagents are the same as those used in the sulfanilic acid procedure.)

Procedure for Tablets and Capsules

Prepare the sample as in the sulfanilic acid procedure.

Development of color is as follows:

Blank	*Standard*	*Blank*	*Sample*
1 ml standard	1 ml standard	1 ml sample	1 ml sample
1 ml buffer	1 ml buffer	1 ml buffer	1 ml buffer
5 ml H_2O	5 ml CNBr	5 ml H_2O	5 ml CNBr
2 ml Tobias acid	2 ml Tobias acid	2 ml Tobias acid	2 ml Tobias acid

Best results are obtained when the sample contains 12–15 mmg of niacin per ml. The development of color is made as follows:

Add the sample, buffer, and water to the tubes. Add 5 ml 10% CN Br from a pipette while the tube is being swirled vigorously. Exactly 30 sec. after the addition of the CN Br add the Tobias acid from a pipette. Swirl the tubes during the addition. (The pipettes for the addition of the CN Br and Tobias acid should be mounted on a ringstand and filled by mechanical suction. The CN Br must be handled under a hood.) After addition of the Tobias acid, close the tubes with rubber stoppers and place in a dark cabinet. Set instrument at 100% transmission at 500 mμ with blank. Read color of sample in 15 min. (The color reaches a peak in about 15 min. and remains at the maximum for the same length of time. If the standard and sample have ca the same niacin content, the concentration is proportional to the photometric density. Otherwise the standard can be run at levels of 8 and 16 mmg/ml and the niacin content of the sample can be obtained by reference to the standard curve. The use of an internal standard has been found to be unnecessary.)

Procedure for Enriched Foods and Food Products

Autoclave the sample with 0.25 *N* H_2SO_4 and precipitate the proteins at the isoelectric point *p*H 4.5, as in the case of the sulfanilic acid procedure. Then pipette 30 ml of the filtrate into a beaker and heat to boiling. Then add drop by drop dilute NH_4OH until the soln smells strongly of ammonia. Continue heating for

2 min; filter; adjust *p*H to 4.5, and transfer to a 50-ml volumetric flask containing 22 g $(NH_4)SO_4$. Dilute to mark, shake vigorously, and filter.

Prepare the standard soln by pipetting two ml of the stock soln at room temp. into a 50-ml volumetric flask and dilute to the mark with H_2O. Then pipette 30 ml of this into another 50 ml volumetric flask containing 22 g $(NH_4)_2SO_4$. Mix and filter. (2-ml aliquots are used for the color development.)

Make up tubes as follows:

Blank	*Standard*	*Blank*	*Sample*
2 ml standard	2 ml standard	2 ml sample	2 ml sample
2 ml buffer	2 ml buffer	2 ml buffer	2 ml buffer
5 ml H_2O	5 ml CNBr	5 ml H_2O	5 ml CNBr
4 ml Tobias acid	4 ml Tobias acid	4 ml Tobias acid	4 ml Tobias acid

DISCUSSION

In order to test the procedures developed, a collaborative study was carried out. Samples of multivitamin capsules, enriched flour, and enriched corn meal were mailed to eight laboratories, which had agreed to collaborate. A supply of Tobias acid was also sent to each laboratory, since we believed that it might not be readily available.

The results obtained by the sulfanilic acid procedure are given in Table 1. The niacin values obtained by the collaborators on Sample 1, multivi-

TABLE 1.—*Nicotinic acid values, using sulfanilic acid procedures*

COLLABORATOR	SAMPLE 1 (MULTIVITAMIN CAPSULES)	SAMPLE 2 (ENRICHED FLOUR)	SAMPLE 3 (ENRICHED CORN MEAL)
	mg/capsule	*mg/lb.*	*mg/lb.*
1	20.8	18.7	19.4
2	21.5	17.2	19.0
3	21.92	16.8	18.4
4	22.19	16.26	18.3
5	21.6	17.1	20.25
6	20.8	16.2	16.0
7	21.3	18.0	20.9
8	20.8	16.5	17.6
Average	21.37	17.06	18.66
Standard Deviation	0.68	0.82	1.42

tamin capsules, agree very closely. The average value reported by eight workers, from six laboratories, was 21.37 mg niacin per capsule with a standard deviation of 0.68. Seven microbiological determinations made by two analysts, working in the same laboratory, and using the U.S.P. method gave an average value of 21.45 with a standard deviation of 1.02. Their results are shown in Table 2. The average sulfanilic acid value obtained on Sample 2, enriched flour, was 17.06 mg per lb with a standard deviation of 0.82. The average microbiological result on this sample was

TABLE 2.—*Nicotinic acid values using U.S.P. microbiological method*

ANALYST	SAMPLE 1	SAMPLE 2	SAMPLE 3
	mg/cap.	*mg/lb.*	*mg/lb.*
1	21.2	17.6	22.56
	21.2	17.44	23.0
	20.2	17.9	21.8
		17.9	
2	21.8	17.6	22.8
	23.0	18.08	23.0
Average	21.45	17.75	22.63
Standard Deviation	1.02	0.20	0.50

17.75 with a standard deviation of 0.2. The average on Sample 3, enriched corn meal, by the sulfanilic acid procedure, was 18.66 mg per lb with a standard deviation of 1.42. The microbiological average was 22.63 with a standard deviation of 0.50.

Nine collaborators, from seven laboratories, submitted results for the Tobias acid procedure. Results are given in Table 3. On Sample 1 the average was 21.46 mg per capsule with a standard deviation of 0.95. Sample 2, had an average of 19.33 mg per lb, with a standard deviation of 2.67. However, one worker reported a value of 25.6 for this sample. Although this figure is much too high we included it in our calculation.

TABLE 3.—*Nicotinic acid values, using Tobias acid procedure*

COLLABORATOR	(MULTIVITAMIN CAPSULES)	(ENRICHED FLOUR)	(ENRICHED CORN MEAL)
	mg/cap.	*mg/lb.*	*mg/lb.*
1	21.2	18.4	24.3
2	21.3	17.6	21.1
3	22.30	20.1	26.55
4	21.96	19.24	22.96
5	21.2	19.8	27.35
6	23.0	25.60	19.70
7	21.7	18.4	21.4
8	19.7	15.9	19.0
9	20.8	18.9	21.3
Average	21.46	19.33 *(18.54)	22.63
Standard Deviation	0.95	2.67 *(1.33)	2.91

* Value of 25.60 not included.

If this result is not included the average becomes 18.54 with a standard deviation of 1.33. The average for Sample 3 was 22.63 mg per lb with a standard deviation of 2.91. The average result obtained on corn meal, by the Tobias acid procedure, 22.629, agrees more closely with the microbiological average 22.632 than does the sulfanilic acid value, which was 18.81. On the other hand, the Tobias acid standard deviation 2.91, on this sample, is greater than that of the sulfanilic acid, 0.82.

On Samples 1 and 2, the average values and standard deviations obtained by sulfanilic acid procedure are less than those given by the Tobias acid procedure. The differences on these samples are, however, not very great.

The results of the collaborative study indicate that the sulfanilic acid procedure is easier to carry out than is the Tobias acid procedure. This is demonstrated by the fact that in every case the standard deviation values reported by the sulfanilic acid procedure are less than the corresponding deviations obtained by the Tobias acid procedure. However, in our hands, if proper precautions are taken to protect the solutions from light, no difficulty is experienced with the Tobias acid procedure. There is a possibility that this procedure might be superior to the sulfanilic acid procedure for the analysis of corn flakes, puffed wheat, and similar products that produce highly colored extracts. However, this point needs further study.

Some of the collaborators commented that in the analysis of flour and corn meal by the sulfanilic acid procedure, the color produced was quite unstable. It is possible to produce a more stable, but less intense color, by the sulfanilic acid procedure if a phosphate buffer is used. Directions for the use of a phosphate buffer in the determination of niacin, by the sulfanilic acid procedure, were therefore sent to each of the collaborators. Some of the workers commented that they found the modified procedure more satisfactory than the original procedure. However, when this modification is used, it is necessary to carry out the color development immediately after the precipitation of the proteins, otherwise low values will be obtained. Some of the workers checked this point by allowing an aliquot of the filtrate from the second protein precipitation to remain in the refrigerator overnight before making a second analysis. In each case a lower niacin value was obtained when compared with the result obtained when the analysis was carried out without interruption. However, if the original instructions are used, the niacin values obtained do not change, even though the filtrate is stored for several days in the refrigerator before the color development is made.

Some of the collaborators felt that the Tobias acid procedure was more difficult to carry out than was the sulfanilic acid procedure. On the other hand, some believed that the greater stability of color, produced by the Tobias acid procedure, was an advantage. One worker commented that Tobias acid does not produce the "typical yellow niacin color." However,

as already mentioned, Tobias acid was selected purposely because it does not produce a yellow color. A yellow niacin color is not desirable because of interference from the blank.

One collaborator remarked that the Tobias acid color seemed to increase slowly for a period of about an hour. He suggested that the readings be taken at a stated time interval, rather than at the peak. Since the method was sent out we have noted that an interfering color develops when the Tobias acid is exposed to the direct rays of the sun. Diffused light does not appear to have this effect, in the time interval required for the color to reach a maximum (about 20 minutes). However, in order to avoid any possibility of light interference, the solutions should be placed in a dark cabinet, immediately after the addition of the Tobias acid, and allowed to remain there until the readings are taken, twenty minutes later.

RECOMMENDATION*

It is recommended that the chemical method for nicotinic acid, using the sulfanilic acid procedure, with phosphate buffer modification, be adopted, first action.

REFERENCES

(1) SWEENEY, J. P., HALL, W. L., and HAGGERTY, J. F., "An Improved Chemical Method for the Determination of Nicotinic Acid," Div. Biological Chemistry, 116th Meeting American Chemical Society, Atlantic City, N. J., 1949.
(2) SWEENEY, J. P., "A Study of the Color Reactions between Cyanogen Bromide and Pyridine Derivatives," Ph.D. Thesis, 1951 (unpublished).

REPORT ON CAROTENE

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

In this year's work efforts were focused upon (1) calibration and checking of instruments (2) adsorbents for carotene (3) purity of solvents, and (4) continuation of the monthly check sample.

Standard for instruments.—A solution of commercial carotene in mineral oil, stabilized with additions of nordihydroguairetic acid and citric acid, was prepared in our laboratory. Tests at elevated temperatures indicated that the carotene should be expected to remain stable for a month or more under ordinary laboratory conditions. Samples were sent to 81 collaborators and results obtained from 60. The mean value obtained by the different collaborators who calculated results with the formula was 1000 mg/lb, and by those who used a calibration curve, 1009 mg/lb. A comparison of results obtained with the different instruments is given in Table 1.

* For report of Subcommittee A and action of the Association, see *This Journal*, **34**, 44 (1951).
† The assistance of E. D. Schall is gratefully acknowledged.

TABLE 1.—*Analyses of standard carotene solution*

INSTRUMENT	NUMBER OF COLLABORATORS	RANGE OF VALUES	STANDARD DEVIATION FROM 1000 MG/LB
Beckman	34	885–1115	48
Coleman	11	871–1152	89
Evelyn	7	862–1110	75
Klett	4	879– 996	73
Others	7	810–1181	130

A second oil sample containing a much lower concentration of carotene was sent to collaborators a month later. The results confirmed those obtained with the first sample in nearly all cases, *i.e.*, those reporting high values were again high and those reporting low values were again low, and to about the same degree. In a few cases, collaborators had evidently readjusted their instruments or otherwise corrected errors after receiving the results on the first oil sample. While it would seem reasonable that all laboratories should be able to check each other within 2 or 3% on such oil samples, the results indicated that a considerable number were consistently outside this range.

Although these instrument errors contribute to the variations which have been observed in the alfalfa meal check samples, it seems doubtful that they are the main source of error, since no consistent pattern was obtained when the carotene in oil values were plotted on coordinate axes with values reported for alfalfa meal sample number 13.

Adsorbents. To obtain a comparison of different lots of magnesia 2642 currently in use in the various laboratories eight collaborators who had reported extremely high or low results on alfalfa meal check sample number 5 were asked to send us a portion of the adsorbent which they used. These different portions were then tested in columns under identical condi-

TABLE 2.—*Differences in adsorbents*

COLL. NO.	CAROTENE REPORTED IN SAMPLE NO. 5	CAROTENE FOUND WITH SAME ADSORBENT IN OUR LABORATORY	MOISTURE CONTENT OF ADSORBENT
	mg/lb.		*per cent*
13	25.0	123	1.03
27	15.6	123	0.90
33	25.6	134	—
50	25.2	—*	2.31
56	—	204*	1.30
60	31.7	132	0.67
69	24.8	128	1.42
72	12.4	127	2.25
Our laboratory	17.0	123	1.31

* Did not hold Xanthophyll.

tions in our laboratory. The results showed that most of them were about equally effective in separating the carotene from an alfalfa while two were wholly unsuitable for use (Table 2). Evidently differences were not due to moisture content of the adsorbent.

Marked differences were observed in the rates at which solvents passed through these columns, and on investigation it was learned that some laboratories were mixing magnesia and supercel 1:1 by weight instead of 1:1 by volume as had been intended, but not stated, in writing the directions. Subsequent studies in our laboratory indicated that mixtures of 1:1 by weight gave slightly higher carotene values but that 2:1 (Mg 0:Supercel) by weight could be used to speed up chromatography without increasing the carotene values.

TABLE 3.—*Comparative adsorption of different brands of magnesia*

BRAND	MOVEMENT OF XANTHOPHYLL ZONE	CAROTENE FOUND
	(*cm*)	*mg/lb.*
1	2	51.4
2	2	52.8
3	4	54.8
4	6	56.5
5	7	56.6
6	9½	61.2
7	10*	—
8	10*	—
9	10*	—

* Xanthophyll obviously passed into eluate.

At various times collaborators suggested the use of a larger volume of eluant since they had observed that more pigment could thus be eluted. Studies in our laboratory confirmed this. However, when the pigment obtained with additional eluant was studied spectrophotometrically the adsorption curve was found to differ considerably from that of *beta*-carotene.

In an effort to find an alternative adsorbent nine different lots of magnesia obtained from seven chemical manufactures were mixed with supercel and used in chromatographing an alfalfa meal extract. All of the samples gave higher carotene values than No. 2642 (Number 1 in Table 3) which is currently used in the official method.

Solvents. Since alcohol is known to be a powerful eluant for carotenols and since it also occurs as an impurity in acetone, collaborators were asked to check their acetone for the presence of alcohol when the first oil sample was sent out. The procedure[2] was as follows:

Prepare the reagent by dissolving 0.5 g $K_2Cr_2O_7$ in 100 ml of concentrated (60–70%) nitric acid. To 3 ml of the reagent in a test tube, add 1 ml of the acetone,

[2] Agulhon, Bull. Soc. Chim., 9, 881 (1911); Chemical Abstracts, 6, 204 (1912).

mix, and note whether a blue color develops within 15 min. In the presence of ca 0.5% or more of alcohol the blue color develops immediately, but as the amount present decreases the length of time for color development increases.

Only two collaborators found alcohol to be present in substantial quantity. Both had been obtaining high results in the analysis of alfalfa meal check samples.

Monthly check sample.—The alfalfa meal check samples were continued, a total of fourteen having been analysed to date. A total of 81 laboratories are currently receiving the sample. While some of the laboratories consistently obtain results close to the average, some others consistently obtain either high or low values and others are variable. The reasons for these differences have not yet been fully revealed. A gradual improvement seems to be evidenced, however.

RECOMMENDATIONS*

It is recommended that studies on carotene analysis be continued.

REPORT ON COAL-TAR COLORS

By KENNETH A. FREEMAN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS†

Acetates, Carbonates, Halides, and Sulfates in Certified Coal-Tar Colors

The Referee recommends that the title of this topic be changed to "Inorganic Salts in Certified Coal-Tar Colors," and that the topic be continued.

Ether Extracts in Coal-Tar Colors

The Referee recommends that the topic be continued.

Halogens in Halogenated Flouresceins

The Referee recommends that the topic be continued.

Identification of Coal-Tar Colors

The Referee recommends that the topic be continued.

Volatile Amine Intermediates in Coal-Tar Colors

The Referee recommends that this topic be continued.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 34, 44 (1951).
† For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951).

Unsulfonated Phenolic Intermediates in Coal-Tar Colors

The Referee concurs with the recommendation of the Associate Referee that the proposed method for β-naphthol in D&C Red No. 9 and D&C Red No. 11 be adopted, first action. The Referee further concurs in the recommendation that the method for β-naphthol in D&C Red No. 35 and D&C Orange No. 17 be revised as given in the Associate Referee report. The Referee recommends that the topic be continued.

Sulfonated Amine Intermediates in Coal-Tar Colors

The Referee recommends that the topic be continued.

Non-Volatile Unsulfonated Amine Intermediates in Coal-Tar Colors

The Referee recommends that this topic be continued.

Intermediates Derived from Phthalic Acid

The Referee concurs with the recommendation of the Associate Referee that the proposed method for FD&C Red No. 3, D&C Orange Nos. 5, 6, 7, 8, 9, 10, 11, 12, 13, and 16; D&C Red Nos. 21, 22, 23, 24, 25, and 26; D&C Yellow Nos. 7, 8, and 9; and Ext. D&C Orange No. 2 be adopted first action. The Referee concurs with the recommendation of the Associate Referee that the proposed method for D&C Yellow No. 10 be adopted, first action. The Referee concurs with the recommendation of the Associate Referee that the proposed method for D&C Yellow No. 11 and D&C Red No. 19 be adopted, first action. The Referee also concurs in the recommendation that the topic be continued.

Mixtures of Coal-Tar Colors for Drug and Cosmetic Use

The Referee recommends discontinuation of this topic.

Lakes and Pigments

The Referee recommends that this topic be continued.

Spectrophotometric Testing of Coal-Tar Colors

The Referee recommends that this topic be continued.

Subsidiary Dyes in FD&C Colors

The Referee concurs with the recommendation of the Associate Referee that the proposed method for lower sulfonated dyes in FD&C Yellow No. 5 be submitted to collaborative study and that this topic be continued.

Determination of Arsenic and Antimony in Coal-Tar Colors.

The Referee concurs in the recommendation of the Associate Referee that the proposed methods for the determination of arsenic and antimony

in coal-tar colors be studied collaboratively, and that the topic be continued.

Boiling Range of Amines Derived from Coal-Tar Colors

The Referee concurs in the recommendation of the Associate Referee that the method for the determination of the boiling range of pseudocumidine obtained from FD&C Red No. 1 be adopted first action, and that the topic be continued.

Determination of Heavy Metals in Coal-Tar Colors

The Referee concurs in the recommendation of the Associate Referee that the proposed procedure for the separation and determination of lead and bismuth in coal-tar colors be submitted to collaborative study. The Referee also concurs in the recommendation that the proposed procedure for the determination of copper in coal-tar colors be submitted to collaborative study. The Referee further concurs in the recommendation of the Associate Referee that the proposed procedure for the determination of cadmium in coal-tar colors be submitted to collaborative study and that the topic be continued.

Hygroscopic Properties of Coal-Tar Colors

The Referee concurs in the recommendation of the Associate Referee that further work on this topic is unnecessary, and that the topic be discontinued.

Sulfonated Phenolic Intermediates in Coal-Tar Colors

The Referee recommends that this topic be continued.

Subsidiary Dyes in D&C Colors

The Referee recommends that this topic be continued.

Intermediates in Triphenylmethane Dyes.

The Referee recommends that this topic be continued.

REPORT ON UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS

By H. HOLTZMAN (*Associate Referee*) and H. GRAHAM
(Ansbacher-Siegle Corporation, Staten Island, New York)

BETA NAPHTHOL IN D&C RED NO. 9

The method of analysis found to be satisfactory for the determination of beta naphthol in last year's work on D&C Red No. 35[1] and D&C Orange No. 17[1] has been successfully applied to the determination of beta naphthol in D&C Red No. 9. This method involves the extraction of the intermediate with petroleum ether, followed by coupling with diazotized sulfanilic acid and titanium trichloride reduction of the dyestuff formed.

Known quantities of purified beta naphthol were added to 10 gram samples of a D&C Red No. 9, and analyses were run by the proposed method, excellent recoveries being obtained. The results are shown in Table 1.

TABLE 1.—*Recovery of Beta Naphthol*

DYE	INTERMEDIATE ADDED	FOUND	INTERMEDIATE RECOVERED
	per cent	*per cent*	*per cent*
D&C Red No. 9	0.00	0.28[1]	—
	0.20	0.49	0.21
	0.20	0.48	0.20
	0.40	0.68	0.40
	0.60	0.89	0.61
	0.80	1.08	0.80
D&C Red No. 11	—	0.31[1]	—
	0.20	0.53	0.22
	0.20	0.54	0.23
	0.40	0.68	0.37
	0.60	0.91	0.60
	0.60	0.90	0.59

[1] Average of a series of four analyses made on the initial sample.

METHOD

The procedure is the same as previously described for D&C Red No. 35[1] except for the following: **(a)** add to paragraph 1, second sentence ". . . and extracted for eight hours at a reflux rate of 180 to 200 drops per minute, return to the thimble"; **(b)** after third paragraph add the following: "NOTE: The dye couplings should be made immediately after extraction is completed, in order to avoid low results."

A commercial sample of D&C Red No. 9 was submitted for collaborative analysis by this method. Results are shown in Table 2. An average

[1] Holtzman, H., and Graham, H., *This Journal*, 33, 387 (1950).

for all the analyses was obtained of 0.24% beta naphthol found. The results obtained by Collaborator No. 2 were considerably lower than the results by all of the other collaborators and may be eliminated. (This collaborator also obtained low values on his analyses of the D&C Red No. 11, reported below.) When these results were eliminated, an average value of 0.26% resulted, with maximum deviations of +0.04% and −0.07%.

TABLE 2.—*Collaborative results*

COLLABORATOR	% BETA NAPHTHOL IN D&C RED NO. 9	DEVIATION FROM AVERAGE*	% BETA NAPHTHOL IN D&C RED NO. 11	DEVIATION FROM AVERAGE
1	0.26	−0.00	0.30	−0.05
	0.25	−0.01	0.33	−0.02
2	0.07	—	0.25	−0.10
	0.07	—	0.23	−0.12
3	0.26	+0.00	0.30	−0.05
	0.30	+0.04	0.32	−0.03
4	0.22	−0.04	0.53	+0.18
	0.22	−0.04	0.22	−0.13
5	0.29	+0.03	0.41	+0.06
	0.29	+0.03	0.36	+0.01
	0.26	+0.00	0.43	+0.08
	0.27	+0.01	0.40	+0.05
6	0.19	−0.07	0.45	+0.10
	0.23	−0.03	0.41	+0.06
Associate	0.28	+0.02	0.30	−0.05
Referee	0.29	+0.03	0.32	−0.03
Average	0.24		0.35	
Average*	0.26			

* Omitting the low value of Collaborator No. 2.

BETA NAPHTHOL IN D&C RED NO. 11

The method used for D&C Red No. 9 was found to be also applicable to D&C Red No. 11.

Known quantities of purified beta naphthol were added to 10 gram samples of a D&C Red No. 11, and analyses were run by the proposed method, excellent recoveries being obtained. The results are shown in Table 1.

A commercial sample of D&C Red No. 11 was submitted for collaborative analyses. The results are shown in Table 2.

An average of the collaborative analyses gave a value of 0.35% beta naphthol found, with maximum deviations of +0.18% and −0.13%. We have obtained beta naphthol values averaging 0.31–0.32% in a number of determinations. The somewhat higher deviations from average shown for the various collaborative analyses are due to the considerably higher value of 0.53% obtained by Collaborator No. 4; this same collaborator, however, obtained the low value of 0.22% in his duplicate determination.

BETA NAPHTHOL IN D&C RED NO. 35
BETA NAPHTHOL IN D&C ORANGE NO. 17

Two possible causes have been found for the low results obtained in last year's collaborative work.[1] It has been found desirable to (a) specify a reflux rate, and (b) to make the dye couplings immediately after extraction of the beta naphthol. Lack of control of these two factors has been observed to decrease the precision of the analyses.

RECOMMENDATIONS*

(1) We recommend that the method for D&C Red, No. 9 and D&C Red No. 11 be adopted, first action.

(2) We recommend that the method for D&C Red No. 35 and D&C Orange No. 17, be revised as follows:

(a) Add to paragraph 1, second sentence " . . . and extracted for eight hours at a reflux rate of 180 to 200 drops per min return to the thimble."

(b) After third paragraph add the following: "Note: The dye couplings should be made immediately after extraction is completed, in order to avoid low results."

The following collaborators cooperated in these analyses:

Calco Chemical Division, American Cyanamid Company, William Seaman reporting; National Aniline Division, Allied Chemical and Dye Corporation, A. T. Schramm reporting; Food and Drug Administration, Cosmetic Division, J. O. Millham reporting; Food and Drug Administration, Cosmetic Division, Keith S. Heine reporting; Food and Drug Administration, Cosmetic Division, Lee S. Harrow reporting; Ansbacher-Siegle Corporation, Emanual Trachman reporting.

* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 46 (1951).

REPORT ON ARSENIC AND ANTIMONY IN COAL-TAR COLORS

By LEE S. HARROW (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

DETERMINATION OF ARSENIC

At the October 1949 meeting of the A.O.A.C., the Associate Referee for the determination of arsenic in coal-tar colors reported the results of an investigation designed to select the best method for the estimation of arsenic in coal-tar colors (1). Comparison of the Gutzeit (2) and Cassil-Wichmann methods (3) indicated that the latter held definitely more promise of successful application to this problem than the Gutzeit method.

The rapid method for arsenic developed by Cassil-Wichmann (3) has been applied to the determination of 5—500 micrograms of AS_2O_3 (3). In a collaborative study of this method, however, the results did not measure up to the expectation of the authors although better precision was achieved than with similar studies using the Gutzeit method (4, 5). A probable cause for the lack of precision in the collaborative studies is the difficulty of exactly duplicating the apparatus of Cassil and Wichmann. In general, for precision (6) analytical methods which depend on an absorption process which is very rigid with respect to absorption time and quantity of reagents, require strict duplication of the absorption apparatus.

Although an apparatus with an absorption component conforming very closely in dimensions to that used by Cassil-Wichmann was employed by the Associate Referee in several recovery experiments, low results were obtained. To test whether or not arsine was being lost from the absorber, a second absorber was placed in series with the first. In subsequent experiments, recoveries in the range of 94–100 per cent were obtained; this is an improvement of 5 to 10 per cent over those obtained with the single absorber. These results are in agreement with those obtained by Klein (15) who previously conducted similar experiments.

The method for the determination of arsenic presented in this paper is that of Cassil-Wichmann, except that two arsine absorbers in series are used instead of a single absorber. This allows greater latitude in dimensional construction of the apparatus.

The samples of coal-tar colors are prepared by "wet ashing" and the arsenic determined in the resulting solution.

EXPERIMENTAL PROCEDURE

All reagents should be As free.

DIGESTION REAGENTS

Sulfuric acid.—Use a C.P. concentrated acid.

Nitric acid.—Use a C.P. concentrated acid.

Perchloric acid.—Use a C.P. concentrated acid.

Ammonium oxalate soln.—Saturated soln.

ISOLATION REAGENTS

Zinc.—Use an analytical grade of 20 or 30-mesh granulated zinc. It should be as free from arsenic as possible, because the arsenic in the zinc predominately determines the size of the blank.

Potassium iodide soln.—Dissolve 15 g of recrystallized KI in water and dilute to 100 ml.

Stannous chloride soln.—Dissolve 40 g of $SnCl_2 \cdot 2H_2O$ in 100 ml of conc. HCl.

Absorbing soln.—Dissolve 3.2 g of $HgCl_2$ (recrystallized from water) and 0.10 g of finely powdered U.S.P. gum arabic in H_2O and dilute to 200 ml.

Lead acetate soln.—Dissolve 10 g $Pb(C_2H_3O_2)_2$ in 80 ml of H_2O, add sufficient acetic acid to have the soln just acid to litmus paper, and make to 100 ml with H_2O.

TITRATING REAGENTS

Buffer soln.—Dissolve 10 g $Na_2HPO_4 \cdot 12H_2O$ in water and dilute to 100 ml.

Standard iodine soln.—Prepare ca 0.05 *N* stock soln of I_2 by dissolving 6.35 g of pure I_2 and 12.7 g KI in a small quantity of water; filter, and dilute the filtrate to 1 liter. Prepare ca 0.005 *N*, 0.001 *N*, and 0.0005 *N* iodine solns by diluting the stock soln. An additional amount (25 g/liter) of KI is added to each of the dilute I_2 solns.

Standard arsenic solns.—Prepare a stock soln by dissolving 1 g of standard As_2O_3 in 25 ml of 20 per cent NaOH soln. Saturate the soln with CO_2 and dilute to 1 liter with freshly boiled water. One ml of this soln contains 1 mg of As_2O_3. Make standard solns containing 100 mmg, 20 mmg, 10 mmg, and 1 mmg per ml by proper dilutions of the stock soln.

Starch indicator.—Weigh 1 g of finely divided 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 a preservative. It may be necessary to prepare solns from several lots of starch in order to obtain a soln that gives a sharp color change under the conditions of the titration.

APPARATUS

The apparatus (Fig. 1A and B) consists of a generator and an absorber system. Glass wool which has been soaked in 10 per cent $Pb(Ac)_2$ soln and thoroly dried is inserted loosely into the trap above the condenser. All connections between the condenser and the absorber should be dry. Particular attention should be paid to cleanliness of the apparatus. All glassware should be washed with dichromate cleaning soln and rinsed with distilled water before use. The delivery tubes of the arsenic isolation apparatus should be immersed in the cleaning soln between runs.

METHOD

DIGESTION AND ISOLATION

Weigh 10 g of color into 800 ml Kjeldahl digestion flask, add 10 ml of conc. H_2SO_4 and 10 ml of conc. HNO_3, and digest on a low flame until the mass begins to clear. Add 15 ml of water and continue the digestion to the clearing stage. Add successive 5 ml portions of conc. HNO_3 until all organic matter is in solution. Introduce slowly 10 ml of a (1+1) mixture of conc. HNO_3 and 60–70 per cent $HClO_4$, and continue the digestion until the mixture in the reaction flask is only lightly colored. Add 5 ml more of the $HClO_4$ mixture, and heat until the initial vigorous reaction

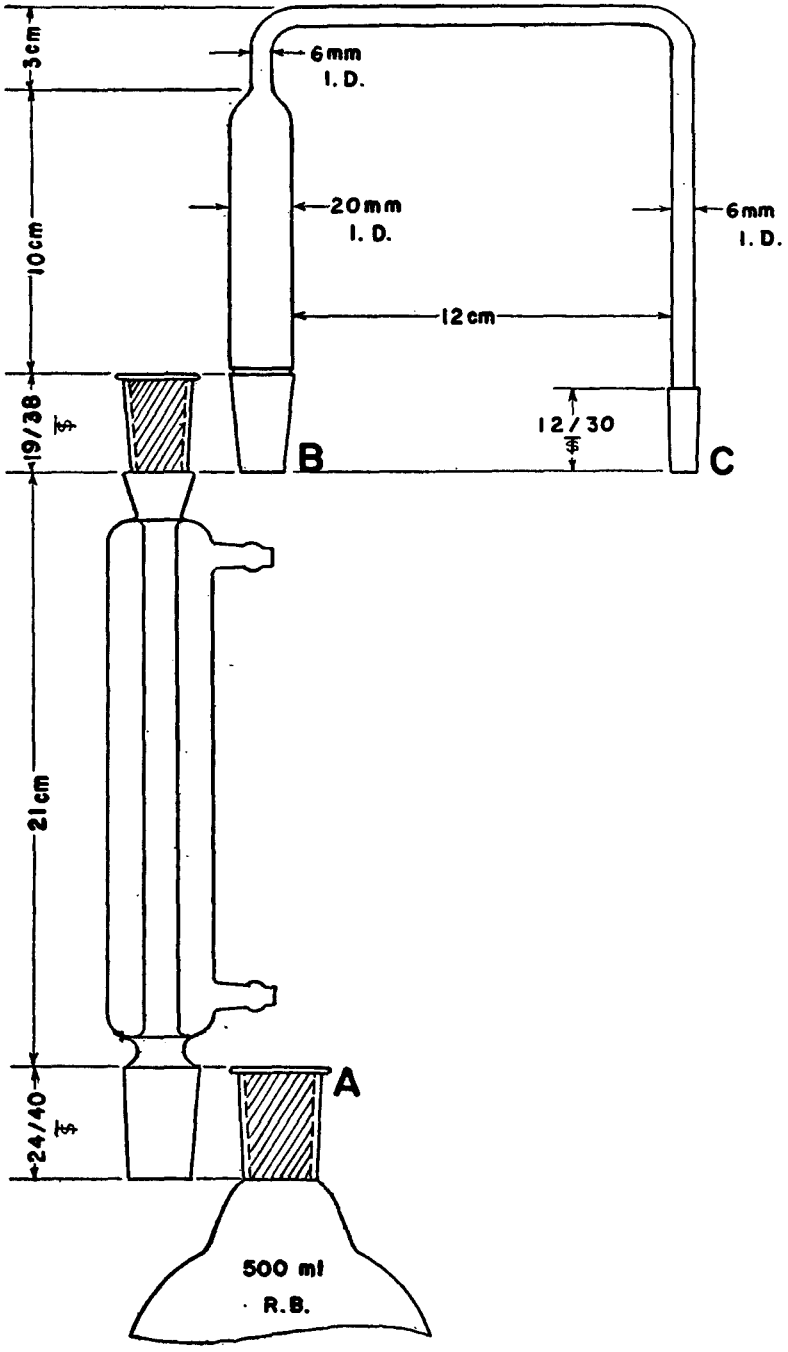

FIG. 1A.—Arsine generator and distilling section. When the arsenic apparatus is in operating position, the absorption section is connected to the distilling section at position C.

subsides. Continue the addition of 5 ml portions of the $HClO_4$ mixture until the digest is water white. Heat until fumes of SO_3 appear, cool, add slowly 20 ml of the ammonium oxalate soln, and again digest until SO_3 fumes appear. Allow the flask to cool and add 50 ml of water. Swirl vigorously and transfer the contents to the arsine generator (A) (see Fig. 1A). Wash the digestion flask with three 20 ml portions of water and add these to the main soln. Add 5 ml of KI soln, 1 ml of $SnCl_2$ soln to the digest and washes, and make the final volume to about 90–100 ml with water. Place 1 ml of absorbing soln in each receiving tube and place in operating position as shown in Fig. 1A. Add 5 g of zinc to the generator, wash down the neck of the flask with a few ml of water, and place in operating position by connecting the flask at (A) (see Fig. 1A).

Heat the generator vigorously until refluxing begins. Lower the flame and continue heating for 12–15 min., then disconnect the first receiver and delivery tube at (C) (see Fig. 1B). Raise the delivery tube, at (D) (see Fig. 1B). Add 2 ml of buffer soln thru the tube, and wash down the outside with 3–5 ml of water delivered in a fine stream. Place the transfer tube* in position at (D) and suck the contents of the second receiver into the first receiver. Wash the outside of the second delivery tube with two 1 ml portions of water, and suck these over into the first receiver. Remove the second receiver (E) and transfer tube. The soln is now ready for titration with iodine and subsequent back-titration with standard As_2O_3 soln.

TITRATION PROCEDURE

Iodine standardization.—In an empty receiver tube place 2 ml of buffer soln and 2 ml of water. Add 3 ml of standard I_2 soln, 5 drops of starch soln, and titrate with standard As_2O_3 soln to a colorless end point. Agitate the soln between additions of the As_2O_3 soln by alternately sucking and blowing thru the stirring tube. (Titrations should be carried out in a good light. The end point can be observed only by looking down through the tube at a white background.)

$$\text{mmg } As_2O_3/\text{ml } I_2 = \frac{\text{mmg } As_2O_3/\text{ml} \times \text{ml } As_2O_3 \text{ soln}}{\text{ml of } I_2 \text{ soln} \times 4}.$$

Unknown titration.—Place the stirring tube in the first receiver and add standard iodine soln slowly until the orange precipitate formed on the addition of the initial portion of the iodine soln disappears. Add 5 drops of starch and titrate with standard As_2O_3 soln to the disappearance of the blue color. Run a blank on all reagents.

$$\% As_2O_3 = \frac{(\text{ml } I_2 \text{ soln} \times I_2 \text{ factor}) - \left(\text{ml } \frac{As_2O_3}{4} \times \text{mmg } As_2O_3/\text{ml}\right) - \text{Reagent Blank}}{\text{Weight of Sample}}.$$

DISCUSSION

The theory of this method for the determination of arsenic is adequately discussed by Cassil-Wichmann (3).

In the wet ashing of the sample, it is essential that all of the organic matter be destroyed without the loss of arsenic oxide. Allcroft, *et al.* (7) and Cassil (5) found that digestion with nitric acid, sulphuric acid, and perchloric acid adequately destroys all organic material with a minimum loss of arsenic. This type of digestion has been applied successfully to

* Any 24/40 standard taper inside joint can serve as suitable transfer tube.

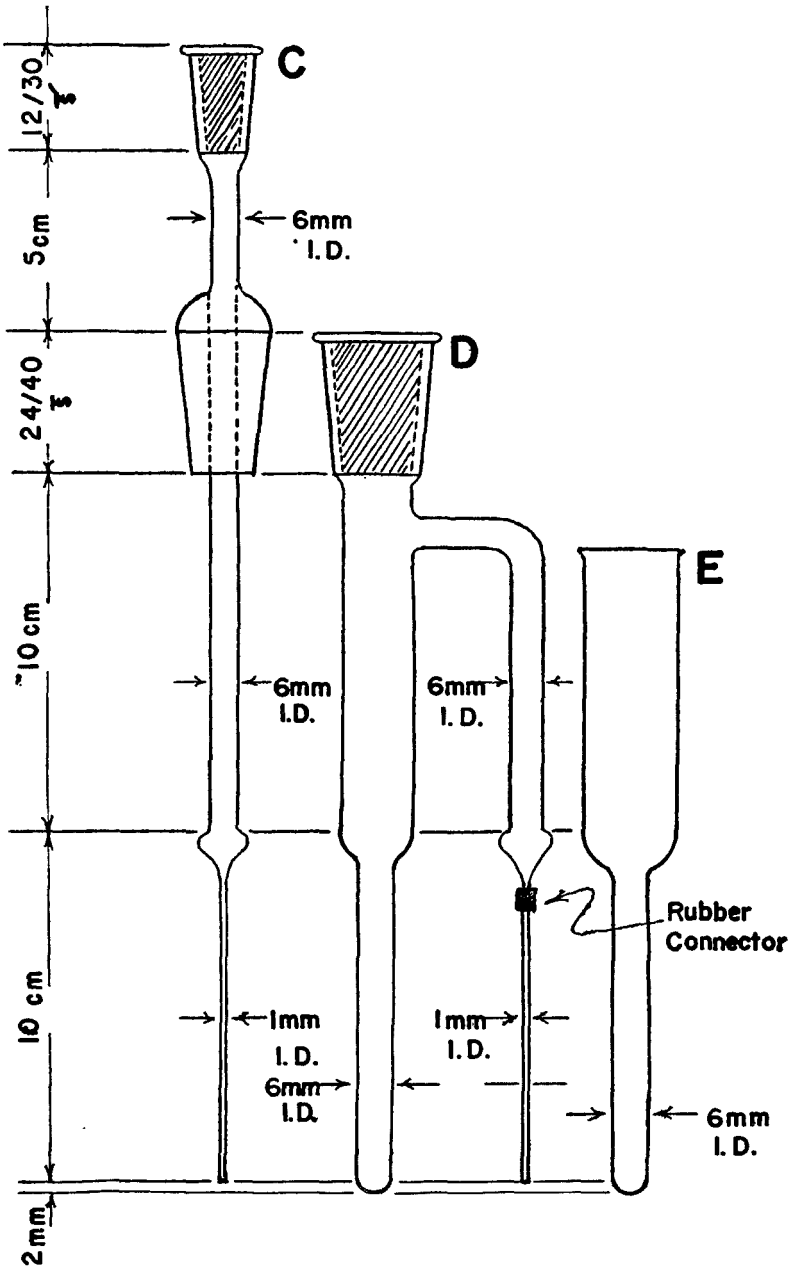

FIG. 1B.—Arsine absorber and titrating tubes.

coal-tar colors for the determination of lead (8, 9). The excess nitric acid must be removed completely at the end of the digestion since the presence of nitrosulphonic acid even in minute amounts makes reduction of arsenic acid to arsenious acid difficult (10). Dilution with water and reconcentration cannot be depended upon to remove nitrogen acids (11) but they can be removed by adding ammonium oxalate solution and heating until sulfur trioxide fumes appear (12).

RESULTS

Known quantities of arsenic were added to portions of a composite sample of FD&C Yellow No. 5, and these portions were analyzed for arsenic by the above method. The results of these experiments are shown in Table 1A. Since the results were satisfactory, the applicability of the method to other coal-tar colors was tested. The results are shown in Table 1B.

TABLE 1A.—*Recovery of arsenic from FD&C Yellow No. 5*

As_2O_3 ADDED	As_2O_3 FOUND GROSS	As_2O_3 FOUND NET*	RECOVERY
mmg	*mmg*	*mmg*	*per cent*
15.00	26.4	13.8	92
15.00	27.1	14.4	96
15.00	26.8	14.2	95
15.00	28.0	15.4	103
5.00	17.6	5.0	100
5.00	17.1	4.4	88
5.00	16.8	4.2	84
10.00	21.9	9.3	93
10.00	21.6	9.0	90
			Av. Rec. 93

* Blank of 12.6 mmg includes the reagent blank plus the arsenic already present in the composite sample.

TABLE 1B.—*Recovery of arsenic from various colors*

COLOR	BLANK	As_2O_3 ADDED	As_2O_3 FOUND GROSS	As_2O_3 FOUND NET	RECOVERY
	mmg	*mmg*	*mmg*	*mmg*	*per cent*
FD&C Yellow No. 3	11.3	15.0	26.8	15.5	103
D&C Red No. 9 Ba Lake	16.1	15.0	30.1	14.0	93
D&C Red No. 21	12.5	15.0	26.7	14.2	95
D&C Red No. 19	16.6	15.0	27.8	11.2	75
D&C Red No. 19	12.0	15.0	25.2	13.2	88
D&C Red No. 34 Ca Lake	13.7	15.0	27.1	13.4	89
				Av. Rec.	90

DETERMINATION OF ANTIMONY

Because antimony forms an hydride in approximately the same degree as arsenic, it would appear that the Cassil-Wichmann method would be suitable for the determination of antimony. Davidson, Cassil, Pully, and Ballard (13) found that SbH_3 is not evolved in any considerable amount when the Cassil-Wichmann procedure is employed for the determination of antimony. Since these workers employed an apparatus having but one absorber, it was decided to check these findings with a double absorber apparatus. The following results were obtained when measured quantities of antimony, added as standard antimonyl potassium tartrate, were tested for recovery by the above method.

TABLE 2.—*Recovery of antimony*

Sb ADDED	Sb RECOVERED	RECOVERY
(*mmg*)	(*gross mmg*)	*per cent*
71.46	1.88	2.6
71.46	2.91	4.1
71.46	2.74	3.8
—*	2.22	—
71.46	1.46	2.0
142.92	12.08	8.4
35.73	1.46	4.1
35.73	2.02	5.5

* Blank.

Monier-Williams (6) postulated that the presence of tin in the generator inhibits the antimony hydride evolution by producing a voltaic type overvoltage, which reduces antimony oxide to antimony metal and stops further reduction. It was, therefore, decided to eliminate tin chloride and potassium iodide from the generator and to use zinc and hydrochloric acid alone as the reduction and evolution reagents. This modification proved

TABLE 3.—*Modified Cassil-Wickmann*

Sb ADDED	Sb RECOVERED	RECOVERY
mmg	*net mmg*	*per cent*
71.5	64.8	91
35.7	32.7	92
28.6	24.1	84
21.4	19.4	90
14.3	13.2	93
35.7	31.4	88
35.7	30.7	86
35.7	32.0	90
		—
		Average 89

satisfactory except for an increased rate of hydrogen evolution. Ballard (14) suggests the addition of 2.5 ml of 1% lead acetate to slow the hydrogen evolution. The results of the above modification were found to be satisfactory for the determination, although there still appears to be some loss of antimony, presumably as the metal. The recovery of antimony by the Cassil-Wichmann procedure, as modified above, is shown in Table 3.

DETERMINATION OF ARSENIC AND ANTIMONY ON THE SAME SAMPLE

An attempt was made to determine arsenic following the procedure proposed above for antimony. The results are shown in Table 4.

TABLE 4.—*Recovery of arsenic*

As added	As recovered	Recovery
mmg	*mmg*	*per cent*
22.9	13.6	59
15.1	14.1	94
20.0	12.5	63
18.8	3.8	2

These experiments show that the two metals must be separated prior to evolution of the hydrides. Davidson, Cassil, and Pully have successfully employed the magnesium orthophosphate precipitation procedure described in the *Book of Methods*. (2) Recoveries of As and Sb separated by magnesium orthophosphate precipitation after digestion are given in Table 5.

TABLE 5.—*Recoveries of arsenic and antimony*

As added	As rec.	As rec.	Sb added	Sb rec.	Sb rec.
mmg	*net mmg*	*per cent*	*mmg*	*net mmg*	*per cent*
15.0	13.6	91	35.7	30.7	86.0
15.0	15.1	100	35.7	32.0	89.7
15.0	13.7	91	35.7	31.3	87.7

Methods have been described for the determination of arsenic and of antimony in coal-tar colors. It is shown that the Cassil-Wichmann method is satisfactory for the determination of arsenic if the apparatus is modified to include two absorbers in series. Antimony may be determined by a modified Cassil-Wichmann procedure in which zinc and hydrochloric acid are substituted for tin chloride and potassium iodide. A preliminary separation of the two metals is necessary for the determination of arsenic in the presence of antimony. The average recovery of arsenic by the Cassil-Wichman procedure was 97%. The average recovery of antimony by the modified Cassil-Wichmann procedure was 90%.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative work be done on the determination of arsenic and antimony in coal-tar colors by the methods proposed above.

(2) The subject be continued.

ACKNOWLEDGEMENT

The author wishes to express his appreciation to H. J. Wichmann and A. K. Klein, of the Division of Food, U. S. Food and Drug Administration, for making the knowledge gained in their experience in determination of traces of metals so freely available in a number of discussions during the course of this work.

The author also wishes to thank Keith S. Heine, Jr., and John O. Millham, of the Division of Cosmetics for their work in preparation of the illustrations used in this paper.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 46 (1951).

REPORT ON BOILING RANGE OF AMINES DERIVED FROM COAL-TAR COLORS

By LEE S. HARROW (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the annual meeting of the Association last year, the results of a collaborative study on a method for the determination of the boiling range of pseudocumidine obtained by reduction of FD&C Red No. 1 (1) were reported (2). Since the evaluation of the "initial boiling point" was not consistent, it was recommended that the method be studied further.

During the past year, the boiling range distilling apparatus was modified to include a stopcock in the takeoff side-arm and the procedure modified to provide a 2-minute total reflux period at the start of the distillation in order that a more constant "initial boiling point" might be obtained.

This modified method for pseudocumidine is as follows:

METHOD

APPARATUS

Steam distillation.—A three-liter, two-neck, round-bottom flask; one neck is fitted with a dropping funnel; the other with a steam distillation trap which has an inlet for live steam and which is connected to a water-cooled condenser.

Fractionating apparatus.—A 25-ml round-bottom flask fitted with an insulated distilling column 10–12 inches long and ½ inch inside diameter, packed with 24 inches of ca 24 gauge nichrome wire coiled inside the column in the manner described by Podbielniak (3). An insulated Vigreau column with a takeoff valve in the side-arm of approximately the same dimensions is also suitable. Provide the column with a 200–260°C. Anschutz thermometer.

REAGENT

Sodium hydrosulfite soln.—A saturated soln of reagent grade $Na_2S_2O_4$ (ca 30%).

DETERMINATION

Dissolve ca 100 g of color in 2 liters of hot water contained in the round-bottom flask of the steam distillation apparatus. Add 10 g of sodium hydroxide and heat the soln to boiling, then while passing live steam thru the soln at a rate that will produce 5–10 ml of distillate per min., add the sodium hydrosulfite soln dropwise by means of the dropping funnel until the red color disappears (the soln will then be a light yellow-brown). Continue the steam distillation until no more oil distills. Extract the distillate with four 20-ml portions of ether, and wash the combined extracts several times with 10-ml portions of water. Evaporate the major portion of the ether on a steam bath and dry the residue over sodium or potassium hydroxide pellets. Filter the residual soln into the 25-ml round-bottom flask of the fractionating apparatus. Heat cautiously with a heating mantle or a water bath until all the ether has been removed; then continue heating with a Wood's metal bath or an equivalent constant temp. bath. Reflux the material for 2 min. after the vapors reach the top of the column, then distill at a rate of about one drop per second. The initial boiling point is the temp. at the end of the 2-min. reflux period. The final point is taken as the maximum temp. at which any material distills.

A composite sample of FD&C Red No. 1 was prepared and samples of this material submitted to the following collaborators:

Charles H. Brown, Bates Chemical Company, Inc.
A. K. Klein, Division of Food, U. S. Food and Drug Administration
W. H. Kretlow, Wm. J. Stange Company
John O. Millham, Division of Cosmetics
A. T. Schramm, National Aniline Division, Allied Chemical and Dye Corporation

The results in the order reported are shown in the following table.

COLLABORATOR	BOILING RANGE FOUND
	°C.
1	224.5–238
2	221.5–235
3	222.2–240
4	223.8–234.5
5	225.3–240.6
	226.0–240.8
	224.0–240.2
Associate Referee	223.9–239.5
	224.5–237.6
	223.8–238.4

RECOMMENDATIONS*

It is recommended—

(1) That the method be adopted, first action.

(2) That the subject, Boiling Range of Amines Derived From Coal-Tar Colors, be continued.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 46 (1951).

REPORT ON INTERMEDIATES DERIVED FROM PHTHALIC ACID

By CHARLES GRAICHEN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

During the past year, the Associate Referee has developed methods for the determination of phthalic acid in D&C Yellow No. 10, and in D&C Yellow No. 11 and D&C Red No. 19. These two methods and the procedure for the determination of phthalic acid in fluorescein and the halogenated fluoresceins which was reported last year[1] have been studied collaboratively.

PHTHALIC ACID IN D&C YELLOW NO. 10

This procedure consists of extracting the phthalic acid from an acid solution of the color with diethyl ether, extracting the phthalic acid from the ether into dilute base, and spectrophotometric determination of the phthalic acid. The extract from the sample used for the recovery experiments showed ultraviolet absorption at 230 mμ, 262 mμ, and 276 mμ, equivalent to 0.05 per cent phthalic acid. From the shape of the entire ultraviolet absorption curve, it appears that very little of this absorption is due to phthalic acid. It may be expected that results of analyses by this method will be slightly high due to the "background" error.

METHOD

REAGENTS

Sodium hydroxide, 1% (w/v).—Dissolve 10 g of NaOH in water and dilute to one liter.

Hydrochloric acid (1+199).—Dilute 10 ml of concentrated HCl to one liter.

Diethyl ether, C.P.

Standard phthalic acid solution.—Accurately weigh 0.15 to 0.18 g of C.P. potassium acid phthalate, dissolve in water, and dilute to exactly 500 ml. Dilute 10 ml of this soln to 200 ml with 0.1 *N* HCl. Calculate the concentration of phthalic acid in this soln as follows:

$$\text{Conc. phthalic acid (mg./100 ml.)} = \text{mg. of } KHC_8H_4O_4 \times 0.00813$$

DETERMINATION

Dissolve a 1-g sample of the color in water and wash into a continuous extractor. Add ca 1 ml of conc. HCl per 100 ml of soln and extract for 8 hours with ca 250 ml of diethyl ether. Transfer the ether to a separatory funnel. Rinse the extraction flask with two small portions of ether and add the washings to the main extract. Wash the ether extract with four 10-ml portions of (1+199) HCl. Combine the washings in a separatory funnel and extract with 50 ml of ether. Combine the ether fractions and extract four times with 10 ml of 1% NaOH soln. Collect the alkaline washes in a beaker and evaporate to dryness. Dissolve the residue in distilled water and transfer to a 200 ml volumetric flask. Add 2 ml of conc. HCl and dilute to

[1]Graichen, Charles, *This Journal*, 33 ,398 (1950).

volume. Proceed as directed in the method for the determination of phthalic acid in fluorescein (1) beginning with "Filter through a dry filter paper . . . "

Known amounts of potassium acid phthalate were added to 1-gram samples of the color and the phthalic acid determined by the proposed procedure. An average recovery of 95% was obtained with concentrations up to 2% phthalic acid. The data are given in Table 1.

TABLE 1.—*Recovery of phthalic acid from D&C Yellow No. 10*

COLLABORATOR	PHTHALIC ACID		RECOVERY*
	ADDED	FOUND	
	per cent	*per cent*	*per cent*
1	—	0.050	—
2	0.163	0.212	99
3	0.407	0.420	91
4	0.815	0.838	95
5	2.03	1.96	94

* Corrected for "blank."

The averages of the results obtained by the Associate Referee and by the collaborators are shown in Table 2. Sample B is sample A with 0.51% phthalic acid added.

TABLE 2.—*Collaborative analysis of phthalic acid in D&C Yellow No. 10*

COLLABORATOR	SAMPLE A PHTHALIC ACID	SAMPLE B PHTHALIC ACID
	per cent	*per cent*
1	0.12	0.62
2	0.15	0.56
3	0.15	0.62
4	0.02	0.58
5	0.14	—
Associate Referee	0.15	0.61

PHTHALIC ACID IN D&C RED NO. 19 AND D&C YELLOW NO. 11

In this procedure the phthalic acid is extracted with dilute alkali from a chloroform solution of the dye, and then determined spectrophotometrically.

REAGENTS

Sodium hydroxide, 1% (w/v).—Dissolve 10 g of NaOH in water and dilute to one liter.

Sodium hydroxide, 10% (w/v).—Dissolve 100 g of NaOH in water and dilute to one liter.

Hydrochloric acid (1+9).—Dilute 100 ml of conc. HCl to one liter.

Chloroform, U.S.P.

Standard phthalic acid soln.—Accurately weigh 0.15 to 0.18 g C.P. potassium acid phthalate, dissolve in water, and dilute to exactly 500 ml. Dilute 10 ml of this soln to 200 ml with ca 0.1 *N* HCl.

$$\text{Conc. phthalic acid (mg/100 ml)} = \text{mg of } KHC_8H_4O_4 \times 0.00813.$$

DETERMINATION

(*a*) *D&C Red No. 19.*—Weigh a 0.5-g sample into a beaker and dissolve in 20 ml of hot water. Cool to room temp and transfer the soln to a 125-ml separatory funnel. Rinse the beaker with 5 ml of H_2O and add the wash water to the funnel. Add 80 ml of chloroform, 2 ml of 10% NaOH, and shake vigorously for one min. Draw off the chloroform layer and wash the aqueous phase twice with 30 ml portions of chloroform, discarding the chloroform. Add 7 ml of (1+9) HCl and wash with two 30-ml portions of chloroform, discarding the chloroform. Transfer the aqueous soln into a beaker, rinse the funnel with 10 ml of distilled water, and transfer to the same beaker. Evaporate to dryness on a steam bath with the aid of an air jet. Proceed as directed in the method for the determination of phthalic acid in fluorescein (1) beginning with "Dissolve the residue in distilled water . . . "

(*b*) *D&C Yellow No. 11.*—Wash a 0.5-g sample into a 125-ml separatory funnel with 80 ml of chloroform. Add 20 ml of 1% NaOH. Proceed as directed above under (a) "D&C Red No. 19," beginning with "Shake vigorously for one minute, line 4."

Known amounts of potassium acid phthalate were added to 0.5-gram samples of the two colors and these samples analyzed by the proposed procedure. Known amounts of the phthalate were also carried through the procedure with no dye present. The average recovery without dye present was 96%, with D&C Yellow No. 11 present 93%, and with D&C Red No. 19 present 86%. The results are shown in Table 3.

TABLE 3.—*Recovery of phthalic acid*

DYE	PHTHALIC ACID		RECOVERY*
	ADDED	FOUND	
	per cent	*per cent*	*per cent*
None	—	0.004	—
None	0.163	0.155	93
None	0.407	0.416	101
None	0.813	0.771	94
D&C Red No. 19	—	0.062	—
D&C Red No. 19	0.163	0.201	85
D&C Red No. 19	0.407	0.422	89
D&C Red No. 19	0.813	0.743	84
D&C Yellow No. 11	—	0.055	—
D&C Yellow No. 11	0.163	0.198	88
D&C Yellow No. 11	0.407	0.430	92
D&C Yellow No. 11	0.813	0.870	100

* Corrected for "blank."

The averages of the results obtained by the Associate Referee and by the collaborators are shown in Table 4. Sample B is sample A with 0.49% added phthalic acid.

TABLE 4.—*Collaborative analysis of phthalic acid in D&C Red No. 19*

COLLABORATOR	SAMPLE A PHTHALIC ACID	SAMPLE B PHTHALIC ACID
	per cent	*per cent*
1	0.05	0.49
2	0.06	0.48
3	0.07	0.53
4	0.05	—
5	0.16	0.55
Associate Referee	0.06	0.51

COLLABORATIVE ANALYSIS FOR PHTHALIC ACID IN D&C YELLOW NO. 8

The averages of the results obtained by the Associate Referee and by the collaborators are shown in Table 5. Sample B is sample A with 0.45% added phthalic acid.

TABLE 5.—*Collaborative analysis of phthalic acid in D&C Yellow No. 8*

COLLABORATOR	SAMPLE A PHTHALIC ACID	SAMPLE B PHTHALIC ACID
	per cent	*per cent*
1	0.24	0.46
2	0.34	0.77
3	0.34	0.71
4	0.33	0.69
5	0.30	0.65
Associate Referee	0.35	0.71

These studies show that the methods do not give great precision or accuracy. They are, however, satisfactory for the determination of phthalic acid in the amounts ordinarily found in the colors studied.

ACKNOWLEDGMENT

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G. Ochek, Calco Chemical Division, American Cyanamid Company
J. F. Walton, H. Kohnstamm and Company, Inc.

RECOMMENDATIONS*

It is recommended—

(1) That the procedure for the determination of phthalic acid in fluorescein and halogenated fluoresceins be adopted as first action.

(2) That the procedure for the determination of phthalic acid in D&C Yellow No. 10 be adopted as first action.

(3) That the procedure for the determination of phthalic acid in D&C Red No. 19 and D&C Yellow No. 11 be adopted as first action.

(4) That the topic be continued.

No reports were given on spectrophotometric testing or on subsidiary dyes in D&C colors.

REPORT ON SUBSIDIARY DYES IN FD&C COLORS—LOWER SULFONATED DYES IN FD&C YELLOW NO. 5

By MEYER DOLINSKY (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the annual meeting of the A.O.A.C. last year, several tentative procedures for the determination of subsidiary dyes in FD&C colors were deleted. This action was taken not because the procedures were known to be faulty, but because there was no evidence that the methods had been investigated with sufficient thoroughness to merit retention.

One of the methods (1) deleted at last year's meeting was that for the determination of lower sulfonated dyes in FD&C Yellow No. 5. This method involves extraction of the color from acid solution into amyl alcohol; removal of the FD&C Yellow No. 5 from the amyl alcohol by washing with 0.25 *N* hydrochloric acid and determination of the residual lower sulfonated dye by titration with titanium trichloride or by colorimetric comparison with a solution of FD&C Yellow No. 5. It was decided to check this method, using purified samples of the possible lower sulfonated dyes and purified FD&C Yellow No. 5.

The two mono-sulfonated colors most likely to be found in commercial samples of FD&C Yellow No. 5 are (1) the disodium salt of 3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole and (2) the disodium salt of 3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole. Both of these subsidiaries have been prepared and the colors, plus a sample of purified FD&C Yellow No. 5, have been used to check the accuracy of the procedure.

Since the spectrophotometric determination of small quantities of a color is more accurate than titration with titanium trichloride the extrac-

* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951).

tion procedure was modified so that the final determination of the subsidiary color was made spectrophotometrically.

EXPERIMENTAL

1-(4-sulfophenyl)-3-carboxy-5-pyrazolone was prepared as described by Fierz-David (2) using phenyl hydrazine p-sulfonic acid and sodium ethyl oxalacetate. 1-phenyl-3-carboxy-5-pyrazolone was prepared in the same manner, substituting phenyl hydrazine hydrochloride for phenyl hydrazine p-sulfonic acid. The sulfonated pyrazolone was recrystallized from (1+1) alcohol$-H_2O$; the unsulfonated pyrazolone was recrystallized from H_2O. Both were dried at 135°C.

The 1-(4-sulfophenyl)-3-carboxy-5-pyrazolone was coupled in alkaline solution with diazotized aniline; the 1-phenyl-3-carboxy-5-pyrazolone was coupled similarly with diazotized sulfanilic acid. After coupling was complete the colors were salted out by the addition of conc. hydrochloric acid filtered off and dried at 135°C. The 3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole (which appears to be more soluble than the other subsidiary) required a large quantity of hydrochloric acid for salting out and was recovered as the color acid. The 3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole was readily obtained as the monosodium salt by the addition of a small quantity of hydrochloric acid.

Analytical data on the two compounds are shown in Table 1.

TABLE 1.—*Recovery comparisons*

SUBSIDIARY COLOR	MOLECULAR WEIGHT (AS DETERMINED BY TITRATION WITH $TiCl_3$)	CALCULATED MOLECULAR WEIGHTS
3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole	387.2	for $C_{16}H_{12}O_6N_4S$ 388.3
3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole	410.9	for $C_{16}H_{11}O_6N_4SNa$ 410.3

Samples of each subsidiary color were extracted by the former A.O.A.C. procedure. Each color is extracted completely from acid solution into the amyl alcohol and not removed to any appreciable extent by washing with 0.25 *N* hydrochloric acid (Table 2).

A sample of purified FD&C Yellow No. 5[1] and mixtures containing known quantities of FD&C Yellow No. 5 and one of the subsidiaries were then extracted, and the amount of residual dye determined spectrophotometrically (Table 3).

The results indicate that the A.O.A.C. procedure is valid for removing both of the lower sulfonated dyes from samples of FD&C Yellow No. 5.

[1] Prepared by C. Graichen, Division of Cosmetics, Food and Drug Administration, Washington, D. C.

TABLE 2.—*Extraction of lower sulfonated subsidiaries of FD&C Yellow No. 3*

SAMPLE	WEIGHT	NOT EXTRACTED INTO AMYL ALCOHOL	WASHED OUT OF AMYL ALCOHOL BY 3 WASHINGS WITH 0.25 *N* HCl
	mg	*per cent*	*per cent*
3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole	200.0	0	0.4
3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole	17.5	0	0.6
3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole	200.0	0	0.4
3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole	18.7	0	0.0

By substituting a spectrophotometric determination of the subsidiary dye for the titanium trichloride titration, the amount of sample required can be cut in half, reducing the number of acid washings required and increasing the accuracy of the determination.

TABLE 3.—*Extraction of FD&C Yellow No. 5 and FD&C Yellow No. 5 subsidiaries*

FD&C YELLOW NO. 5	3-CARBOXY-5-HYDROXY 1-p-SULFOPHENYL-4-PHENYLAZO-PYRAZOLE	3-CARBOXY-5-HYDROXY-1-PHENYL-4-p-SULFOPHENYL-AZO-PYRAZOLE	SUBSIDIARY RECOVERED
mg	*mg*	*mg*	*per cent*
180	—	15.4	95.7
180	15.4	—	95.8
200	—	—	0.0

SPECTROPHOTOMETRIC DATA[2]

Absorption curves of the lower sulfonated subsidiaries in aqueous solution at various *p*H levels are shown in Figures 1 and 2. The spectrophotometric data is summarized in Table 4.

TABLE 4.—*Spectrophotometric data for FD&C Yellow No. 5 subsidiaries*

SUBSIDIARY	ABSORPTION PEAK	E/MG/LITER (AS DISODIUM SALT)
	mμ	
3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole	433 ± 2—neutral	0.0576
	438 ± 2—0.1 *N* HCl	0.0549
3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole	428 ± 2—neutral	0.0637
	431 ± 2—0.1 *N* HCl	0.0598

[2] Obtained with a G.E. Recording Spectrophotometer with an effective slit width of 8 mμ.

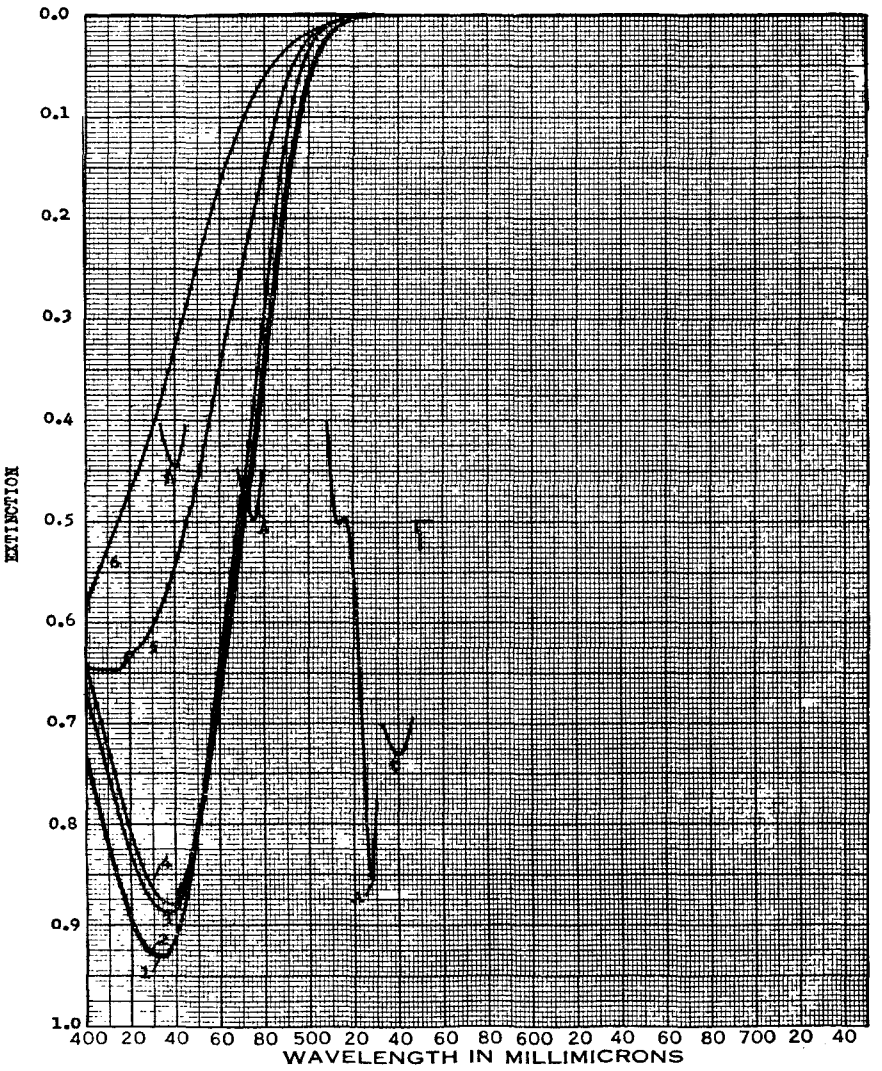

FIG. 1.—FD&C Yellow No. 5—Subsidiary-3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole.

Conc:=15.23 mg/liter
Curve 1—*p*H 6.7
Curve 2—*p*H 3.7
Curve 3—In 0.1 *N* HCl
Curve 4—In 1 *N* HCl
Curve 5—*p*H 9.5
Curve 6—In 0.1 *N* NaOH
A = Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
C = Signal Lunar White Glass H-6946236.

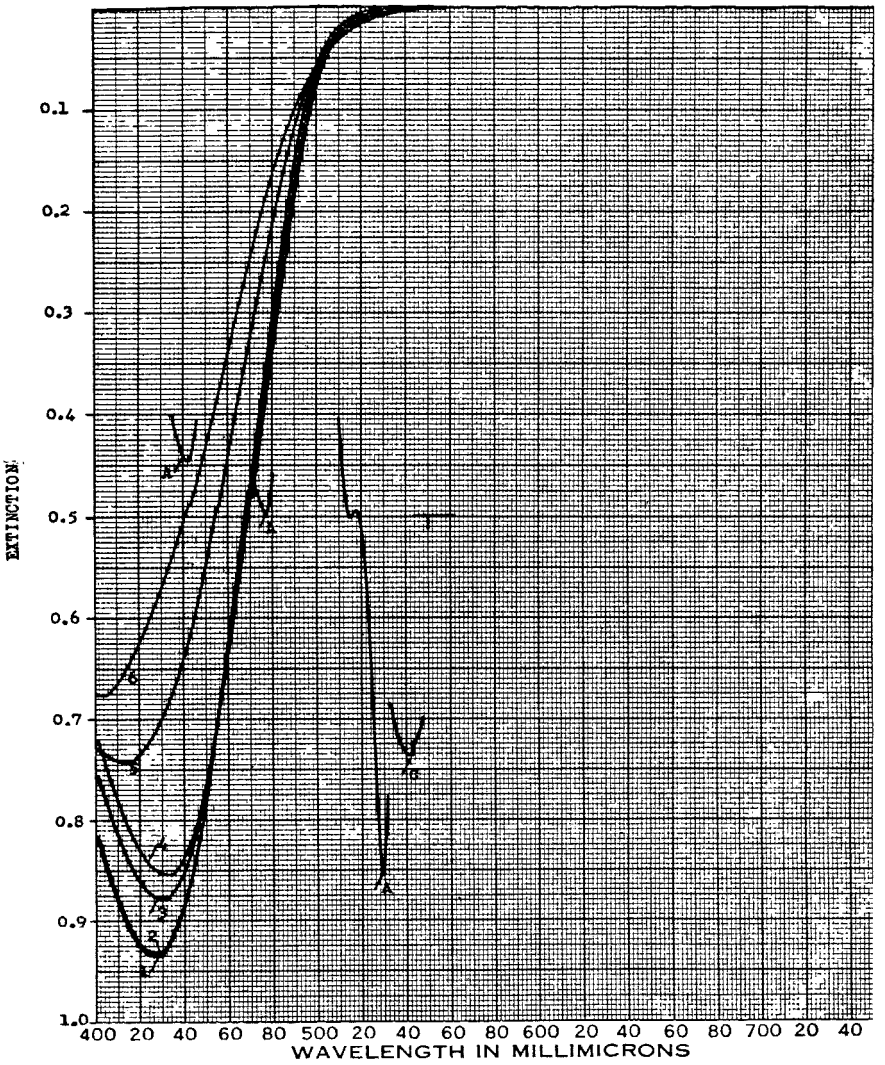

FIG. 2.—FD&C Yellow No. 5—Subsidiary-3-carboxy-5-hydroxy-1-phenyl-4-p-sulfophenylazo-pyrazole.

Conc: = 13.07 mg/liter
Curve 1—*p*H 6.5
Curve 2—*p*H 3.5
Curve 3—In 0.1 *N* HCl
Curve 4—In 1 *N* HCl
Curve 5—*p*H 9.2
Curve 6—In 0.1 *N* NaOH
A = Corning Didymium Glass 512, 6.0 mm.
(Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
C = Signal Lunar White Glass H-6946236.

APPLICATIONS TO COMMERCIAL SAMPLES

Five commercial samples of FD&C Yellow No. 5, representing five different manufacturers, were analyzed for lower sulfonated dyes by the procedure. In each case the amount of subsidiary dye was found to be less than 0.1 per cent. In calculating the percentage of subsidiary present the absorbency per mg. per liter of the 3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole is used as a standard. However, since both subsidiaries have very nearly the same absorbency per mg. per liter, the presence of even a moderate amount of the other subsidiary will not seriously affect the final results. The absorption peak of the extracted subsidiary in 0.1 *N* hydrochloric acid was found to be at 438 ± 2 mμ. This would indicate that the bulk of the subsidiary is 3-carboxy-5-hydroxy-1-p-sulfophenyl-4-phenylazo-pyrazole, which would occur in FD&C Yellow No. 5 through the presence of aniline as an impurity in commercial sulfanilic acid.

SUMMARY

Two mono-sulfonated subsidiary dyes of FD&C Yellow No. 5 have been prepared and their spectrophotometric characteristics determined.

The former tentative A.O.A.C. procedure for lower sulfonated dyes in FD&C Yellow No. 5 has been shown to separate quantitatively both monosulfonated isomers from FD&C Yellow No. 5.

Commercial samples of FD&C Yellow No. 5 contained only very small quantities of lower sulfonated dye, the bulk of which is the subsidiary produced from aniline present in the sulfanilic acid used to manufacture the color.

RECOMMENDATIONS*

It is recommended—

(1) That the procedure for lower sulfonated dyes in FD&C Yellow No. 5 (1) be submitted to collaborative study.

(2) That the procedure be adopted as first action.

(3) That the topic be continued.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945).

(2) FIERZ-DAVID, H. A., and BLANGEY, L., "Fundamental Processes of Dye Chemistry." Interscience Publishers, Inc., New York (1949), p. 128.

* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951).

REPORT ON HEAVY METALS IN COAL-TAR COLORS—CADMIUM

By CHARLES STEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

In recent years a number of papers have been published on the determination of micro quantities of cadmium by the dithizone method. It has been applied successfully to the determination of cadmium in foods, biological materials, and certain minerals. The dithizone method is at present employed for the evaluation of other heavy metals in coal-tar colors and no special equipment is required in the case of cadmium.

The procedure to be described is drawn for the most part from three previously published articles, namely, those of Klein and Wichmann (2), Sandell (4), and Shirley, Benne, and Miller (5). These methods are alike in being based on the original observation by Fischer and Leopoldi (1) that cadmium can be extracted by dithizone from a 5 per cent sodium hydroxide solution.

METHOD

REAGENTS

Hydrochloric acid, nitric acid, sulfuric acid, perchloric acid (72%), acetic anhydride, citric acid, ammonium hydroxide, and chloroform.—C.P. or reagent grade.

Sodium hydroxide soln.—20% w/v.

Hydroxylamine hydrochloride solution, 10% w/v.—Dissolve 100 g of $NH_2OH \cdot HCl$ in about 200 ml of water. Make slightly alkaline with ammonium hydroxide and remove heavy metals by shaking with a $CHCl_3$ soln of dithizone. Remove excess dithizone by successive extractions with $CHCl_3$. Acidify with HCl and dilute to 1 liter.

Thymol blue solution, 0.1%.—Dissolve 0.1 g of thymol blue in water, add sufficient 0.1 *N* NaOH to change the dye to blue, and dilute to 100 ml.

Dimethylglyoxime reagent.—Dissolve 2.0 g of dimethylglyoxime in 250 ml of water containing 10 ml of NH_4OH. Filter and dilute to 1 liter with water.

Carbon tetrachloride.—Reagent grade. (See Discussion.)

Diphenylthiocarbazone (dithizone) in $CHCl_3$.—1 mg of dithizone per ml of $CHCl_3$. Dithizone available at present requires no purification.

Dithizone in CCl_4.—Prepare a stock soln of dithizone containing 200 mg per liter. Store in a refrigerator. Prepare, as required, a dilute soln of 20 mg of dithizone per liter by dilution of the stock soln.

Standard cadmium soln.—Dissolve 1 g of pure Cd in 20–25 ml of (1+9) HNO_3. Evaporate to dryness. Add 5 ml of (1+1) HCl. Evaporate to dryness. Add several ml of water and evaporate to dryness again. Dissolve the $CdCl_2$ in 1 liter of water containing 15 ml of HCl, giving a stock soln of 1000 mmg of Cd per ml. Prepare an intermediate soln of 100 mmg of Cd per ml and a working soln of 5 mmg of Cd per ml in 1.5% of HCl.

Absorbent cotton, metal free.—If traces of metal are present, digest the cotton for several hours with warm 0.2 *N* HCl, filter on a Büchner funnel, wash thoroly with distilled water to remove the acid and dry.

PREPARATION OF SAMPLE

A. Barium and Strontium absent.—Transfer a 1-g sample to a 500-ml Kjeldahl flask. Add 10 ml of H_2SO_4, 10 ml of HNO_3, and heat until the bulk of the HNO_3 and water have been expelled. When necessary add more HNO_3 in small amounts until the dye appears to be nearly destroyed. Add 10 ml of a mixture of equal volumes of HNO_3 and 72% $HClO_4$ and heat strongly until all the $HClO_4$ has been expelled (about 20 min.). Avoid excessive spattering during the course of the digestion. The soln should be colorless or very faintly yellow. If organic matter appears to be present, allow to cool, add more of the $HClO_4$-HNO_3 mixture, and again expel excess $HClO_4$. Prepare two blanks in the same manner, each containing all the reagents used but no sample.

Allow the soln to cool and add 10 ml of water. Cool, add 10 ml of 50% citric acid, and 5 drops of thymol blue soln. Neutralize by adding NH_4OH in small portions with cooling. Add 3–5 ml of $NH_2OH \cdot HCl$ soln. If any solid matter remains, digest the mixture at the boiling point for 20 min.

Transfer the cold soln to a 250-ml separatory funnel (filter, if necessary). Rinse the flask three times with five 10-ml portions of hot water, and add the washings to the separatory funnel.

B. Barium and Strontium present.—Digest a 1-g sample until the dye has been completely destroyed as described in *A*. Heat until all water has been expelled and continue heating for 20 min. or until a clear soln is obtained. Barium and strontium sulfates will dissolve in the concentrated H_2SO_4. (The sulfates of other metals, *e.g.* aluminum, may not dissolve.) Cool the soln thoroly. Add 10 ml of acetic anhydride and mix well. Add 25 ml of water cautiously. (Add the water as soon as possible to avoid excessive decomposition of the $(CH_3CO)_2O$). Digest the precipitated sulfates for 15 min. at the boiling point.

Cool, add 10 ml of 50% citric acid, and 5 drops of thymol blue soln. Neutralize with NH_4OH as in *A*. Add 3–5 ml of $NH_4OH \cdot HCl$ soln. Digest at the boiling point for 20 min. Filter directly into a 250-ml separatory funnel and wash the flask and filter paper 3 times with hot water.

DETERMINATION OF CADMIUM

To the soln from *A* or *B* add 3 drops of thymol blue indicator and make alkaline with NH_4OH, if necessary. Prepare a standard by adding 10 mmg of Cd to one of the blank solns. The second blank soln serves as a reagent blank. Treat samples, standard, and blank as directed below.

Add 15 ml of $CHCl_3$ and 3–4 ml of dithizone soln (1 mg per ml in $CHCl_3$) and shake vigorously for 2 min. If the $CHCl_3$ layer is not greenish at this point, add additional 3-ml portions of dithizone soln, shaking vigorously each time, until the green color indicating excess dithizone appears. Allow the $CHCl_3$ layer to settle for at least 3 min. (The organic phase should be given at least 3 min to separate after each shaking period throughout the procedure.)

Transfer the $CHCl_3$ layer quantitatively to a second separatory funnel containing 40 ml of 2% HCl. Add 15 ml of $CHCl_3$ to the first funnel and shake vigorously for 30 seconds. Transfer the $CHCl_3$ layer to the second funnel.

Shake the second funnel vigorously for 2 min. Drain and discard the $CHCl_3$ layer. Free the soln of dithizone by shaking for 15 second periods with successive 10-ml portions of $CHCl_3$ until the $CHCl_3$ layer is colorless. Discard the $CHCl_3$ washes.

Add 2 drops of thymol blue soln and 5 ml of dimethylglyoxime reagent. Add NH_4OH dropwise until the soln is just blue. Add 10 ml of $CHCl_3$, shake vigorously

for 30 seconds, drain and discard the $CHCl_3$ layer. Repeat the washing with a second 10-ml portion of $CHCl_3$. Discard the $CHCl_3$ layer.

Add 15 ml of 20% NaOH soln and 10 ml of CCl_4 and shake very vigorously for 15 seconds. Remove the cover of the separatory funnel and allow the film of CCl_4 on the surface to evaporate (about 20 minutes). Drain and discard the CCl_4 layer, allowing the aqueous phase to fill the stopcock bore.

Add exactly 25 ml of dithizone soln (20 mg per liter of CCl_4) and shake vigorously for 2 min. Allow 1–2 ml of the CCl_4 layer to pass thru the bore of the stopcock. Dry the stem of the funnel with absorbent cotton and insert a fresh pledget of cotton. Determine the absorbency of the remaining CCl_4 layer at 515 mμ after discarding the first 1–2 ml which passes thru the cotton filter.

$$\text{Cd, micrograms} = \frac{(A_{\text{sample}} - A_{\text{blank}})\ (10)}{A_{\text{standard}} - A_{\text{blank}}}$$

where A represents the absorbency of the indicated solns.

The final extraction of Cd must be carried out in the absence of direct sunlight. Optical measurements must be made within 30 min. after the Cd is extracted.

DISCUSSION

The theory underlying the procedure may be briefly summarized as follows: In the first funnel all metals reacting with dithizone are extracted. In the second funnel the acid-stable dithizonates are separated from the cadmium and other acid-unstable dithizonates and discarded. Of the metals remaining in the aqueous phase, in addition to cadmium, only nickel, cobalt, and zinc constitute likely sources of interference.

Shirley *et al.* (5) have shown that interference by nickel and cobalt is effectively eliminated by dimethylglyoxime. Experience has indicated that nickel and cobalt are apparently not serious interferences in the case of certifiable colors. In routine analyses, considerable time can be saved if, as a first approximation, dimethylglyoxime is not employed. In this case, shake the chloroform solution from the first funnel with 45 ml of 2 per cent hydrochloric acid and proceed as described, omitting the addition of the dimethylglyoxime reagent and the two subsequent chloroform washes.

Shirley and co-workers have also shown that 1000 micrograms of zinc produced interference equivalent to only 0.2 microgram of cadmium. Since this quantity of zinc would correspond to fully 0.1 per cent in a 1-gram sample, interference by zinc is unlikely.

The quality of the carbon tetrachloride employed is of critical importance. It should be tested by the following procedure: Arrange a series of six separatory funnels containing 0, 5, 10, 15, 20, and 25 micrograms of cadmium in 45 ml of water. Add 15 ml of 20 per cent sodium hydroxide. Extract the cadmium with exactly 25 ml of dithizone reagent in carbon tetrachloride and determine the absorbencies as described previously. Plot absorbency *vs.* micrograms of cadmium. All points should fall on or very close to a straight line. If this is not the case, the carbon tetrachloride is unsuitable.

Impure carbon tetrachloride can be purified by the following procedure recommended by Klein (3): Reflux the carbon tetrachloride vigorously for 1 hour with one-twentieth its volume of 20 per cent potassium hydroxide in methyl alcohol. Cool and transfer to a separatory funnel. Drain the carbon tetrachloride and wash free of alkali by shaking with successive portions of water. Dry over calcium chloride, filter, and distill in all-glass Pyrex equipment. This method is effective in purifying carbon tetrachloride, but not very practical when large volumes of the solvent are required. In this work a reagent grade of carbon tetrachloride from one manufacturer proved to be satisfactory, whereas a C.P. brand from another source was unsuitable.

No difficulty was experienced with colors free of metals. When such metals as aluminum, calcium, or zirconium are present, it is important to dissolve all solid material (except clay or talc) completely in the ammoniacal citrate solution. A digestion period of twenty minutes is sufficient. Talc and clay can be separated by filtration without loss of cadmium.

In the case of dyes containing barium some difficulty was encountered. Recoveries of cadmium were variable and low, ranging up to about 3 micrograms at the 20 microgram level. The barium sulfate readily passed through the filter paper.

The introduction of the acetic anhydride modification appears to overcome both difficulties in a reasonably satisfactory manner. The tendency of barium sulfate to retain cadmium apparently depends on the concentrations of both substances present when the barium sulfate is precipitated. The acetic anhydride probably serves primarily as a diluent, since it can be added to the concentrated sulfuric acid solution without precipitating barium sulfate. The digestion with acetic acid gives a precipitate of barium sulfate that is more readily filtered after neutralization with ammonia. Traces of barium sulfate may persist in passing through the filter, but this does not interfere with subsequent steps.

The instability of cadmium dithizonate is well known, particularly when exposed to direct sunlight. Shirley *et al.* resorted to the use of amber glassware to protect the complex. However, they also showed that the same initial transmittance readings are obtained whether plain or amber glassware is used when the measurements are made within one-half hour after the extraction of cadmium dithizonate into carbon tetrachloride. In this work plain glassware was employed. Optical measurements were made with a neutral wedge photometer using a 25-mm cell.

RESULTS

The results of twenty series of recoveries are shown in the accompanying table. In each series, recoveries were made at four levels of cadmium over the range 0–25 micrograms, the appropriate volume of standard cadmium solution being added to the dye and carried through the procedure.

In no case was cadmium found to be present within the apparent limits of accuracy of the procedure. The results obtained in the presence of barium and strontium are slightly, but not seriously, low.

TABLE 1.—*Results on 20 samples*

DYE	SUBSTRATUM	Cd ADDED					
		0	5	10	15	20	25
FD&C Orange No. 1		0.2	4.6	9.9	—	19.7	—
FD&C Blue No. 2		−0.2	4.9	10.3	—	—	25.1
FD&C Red No. 1		0.0	—	10.0	14.8	19.9	—
FD&C Yellow No. 5		−0.1	—	9.7	15.1	19.7	—
D&C Violet No. 1		0.0	5.1	10.2	—	—	25.2
FD&C Yellow No. 4		0.0	4.9	—	14.7	—	25.2
FD&C Green No. 2		−0.1	—	9.7	15.0	19.7	—
Ext. D&C Green No. 1		0.1	—	10.0	14.8	19.7	—
D&C Yellow No. 11		−0.1	—	9.8	14.9	19.5	—
D&C Red No. 19 Al Lake	Al benz. and clay	0.2	—	9.9	15.1	19.9	—
FD&C Red No. 3 Al Lake		0.0	—	10.0	15.1	20.0	—
D&C Red No. 7 Ca Lake	Rosin	0.2	—	10.3	15.6	20.4	—
D&C Red No. 11 Ca Lake	Rosin	0.0	—	10.0	15.3	20.3	—
D&C Yellow No. 6 Al Lake	Al and talc.	0.2	—	10.0	14.7	19.5	—
D&C Yellow No. 5 Zr Lake	Al	0.0	—	10.1	15.2	20.0	—
D&C Red No. 9 Ba Lake	ca 60% gloss white	0.1	—	9.5	14.4	19.3	—
D&C Red No. 35 $BaSO_4$ Lake	ca 7.0% blanc fixe	−0.2	—	9.9	15.1	19.9	—
D&C Red No. 9 Ba Lake	ca 60% gloss white	0.0	—	9.5	14.8	19.2	—
D&C Orange No. 17 $BaSO_4$ Lake	ca 80% blanc fixe	0.0	—	9.5	14.1	18.9	—
D&C Red No. 13 Sr Lake	ca 45% blanc fixe	0.0	—	9.0	14.2	18.9	—

SUMMARY

A dithizone procedure for the determination of cadmium in certifiable coal-tar colors has been described. No cadmium was found in twenty representative samples.

RECOMMENDATIONS*

It is recommended that this procedure be submitted to collaborative study.

REFERENCES

(1) FISCHER, H., and LEOPOLDI, G., *Mikrochim Acta.*, **1**, 30 (1937)
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(5) SHIRLEY, R. L., BENNE, E. J., and MILLER, E. J., *Anal. Chem.*, **21**, 300 (1949).

* For report of Subcommittee B and action of the Association, see *This Journal*, **34**, 47 (1951).

REPORT ON HEAVY METALS IN COAL-TAR COLORS—LEAD AND BISMUTH

By CHARLES STEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

In the procedure currently used to determine lead in coal-tar colors, the lead is extracted from an ammoniacal solution containing cyanide by means of dithizone and finally determined by electrolysis and titration with sodium thiosulfate solution. Possible interference by bismuth has been disregarded (*Methods of Analysis, A.O.A.C.*, 6th Edition, **29.43**).

However, the electrolysis of solutions containing both lead and bismuth has shown that such interference can be serious. Varying amounts of bismuth (ranging from 0 to 1000 mmg) were added to 100 mmg of lead, and the solutions were electrolyzed. The titration values (based on the lead present) were either high or low depending on the ratio of bismuth to lead.

When a solution containing 1000 micrograms of bismuth and 100 micrograms of lead was electrolyzed, the volume of sodium thiosulfate required was about half that required for 100 micrograms of lead in the absence of bismuth. With lower ratios of bismuth to lead the interference was positive, but inconsistent.

This report presents a procedure for eliminating bismuth interference in the lead determination. In addition, colorimetric dithizone procedures for both lead and bismuth are described. The procedure cannot be applied directly to barium lakes, nor has it been tested on dyes yielding insoluble residues upon digestion.

METHOD

REAGENTS

Potassium cyanide.—10% soln.

Citric acid, special grade.—low in lead.

Chloroform.—USP.

Ammonium nitrate soln, 15% w/v.—Make the soln alkaline to litmus with NH_4OH. Remove heavy metals by shaking with a $CHCl_3$ soln of dithizone. Remove excess dithizone by shaking with successive portions of $CHCl_3$.

Dithizone solns.—Prepare a stock soln containing 1 mg per ml in $CHCl_3$. Prepare the required solutions by dilution of the stock solution.

Ammonia-cyanide solns:

A. Dilute 250 ml of NH_4OH and 20 g of KCN to 1 liter.

B. Dissolve 20 g of KCN in 1 liter of NH_4OH.

Standard lead and bismuth solns.—Prepare stock solns containing 1 mg per ml of Pb and Bi in 1% HNO_3. Dilute the stock solns to obtain working standards—10 or 20 mmg per ml for Pb and 20 mmg per ml for Bi. The nitrates of the metals are suitable for standards. $Bi(NO_3)_3$ should be dissolved in conc. HNO_3 before diluting with H_2O.

Other reagents as under cadmium.*

* *This Journal*, page 417.

PREPARATION OF SAMPLE

A. Bromine and iodine absent.—Digest the dye as under A for cadmium* Run two reagent blanks concurrently.

B. Bromine and/or iodine present.—Digest a 1 g sample as in A. Cool. Add 10 ml of H_2O and 2 ml of 10% $NH_2OH \cdot HCl$ soln. Heat to the appearance of strong SO_3 fumes. Cool. Add 10 ml of HNO_3 and take to SO_3 fumes. Run two reagent blanks concurrently.

Cool the digest from A or B. Add 10 ml of H_2O and 20 ml of 50% citric acid soln. Cool and add 4 drops of thymol blue soln. Add NH_4OH in small portions, with cooling, to the appearance of a blue color. Add 5 ml of 10% KCN soln and 5 ml of 10% $NH_2OH \cdot HCl$ soln. Transfer the soln to a 250-ml separatory funnel. Rinse the flask with three 10-ml portions of H_2O and add the washings to the funnel. To one of the reagent blanks add 50 mmg of Pb and 100 mmg of Bi. This is the standard. Carry the second blank as the reagent blank.

DETERMINATION OF LEAD AND BISMUTH

To the soln in the first funnel add ca 10 ml of dithizone soln (50 mg per liter in $CHCl_3$) and shake vigorously for 1 min. (A green or purple color in the $CHCl_3$ layer indicates an excess of dithizone.) If necessary, repeat the extraction with 10 ml portions of dithizone soln until an excess of dithizone is present.

To a second 250 ml separatory funnel add 50 ml of 15% NH_4NO_3 soln and 3 drops of thymol blue soln. Add dilute (approx. 1+5) HNO_3 dropwise to the NH_4NO_3 soln to the appearance of a full red color.

Transfer the $CHCl_3$ layer quantitatively from the first funnel to the second funnel. Wash the stem of the first funnel by passing small portions of $CHCl_3$ thru the funnel, collecting the $CHCl_3$ in the second funnel. Add 10 ml of dithizone soln to the first funnel and shake vigorously for one min. The $CHCl_3$ layer should remain green. If it does not, repeat the extraction with 10 ml. portions of dithizone soln until the $CHCl_3$ remains green. Combine the $CHCl_3$ extracts in the second funnel. Add 10 ml of $CHCl_3$ to the first funnel, shake for 10 seconds, and transfer the $CHCl_3$ to the second funnel. Discard the first funnel.

Shake the second funnel vigorously for 20 seconds. Allow the $CHCl_3$ to settle. Add 3 drops of thymol blue soln. Add dilute HNO_3 dropwise to the re-appearance of a full red color in the aqueous layer. (It is necessary to readjust the *p*H at this point because NH_4OH is somewhat soluble in $CHCl_3$ and is carried over from the first funnel.)

Shake the second funnel vigorously for 2 min. Allow the $CHCl_3$ to settle. The aqueous layer now contains all of the lead and some of the bismuth. Transfer the $CHCl_3$ phase to a third 250-ml separatory funnel containing 50 ml of 5% HNO_3. Add 4 drops of thymol blue soln to the second funnel and adjust the *p*H to 2.5 by adding dilute NH_4OH (approx. 1+20) dropwise. (The desired *p*H is indicated by a very faintly orange tint.) Disregard the red color which appears in the residual $CHCl_3$.

Remove bismuth by shaking vigorously (for one-min. periods) with successive 10 ml portions of dithizone soln (100 mg/liter in $CHCl_3$). Transfer the $CHCl_3$ layer to the third funnel in each case before adding the succeeding portion of dithizone. Repeat until the original green color of the reagent remains completely unchanged. (Bismuth is extracted slowly at this *p*H. A "greenish" color does not indicate the complete removal of bismuth. The color should remain entirely unaltered.) Add 10 ml of dithizone soln and shake vigorously for 2 min. Transfer the $CHCl_3$ layer to the third funnel. Shake the second funnel for 10-second periods with successive 5 ml portions of $CHCl_3$ until both the aqueous and $CHCl_3$ layers are colorless, collecting

the washes in the third funnel. Allow the final (colorless) portions of $CHCl_3$ to remain in the second funnel.

Shake the third funnel vigorously for 1 min. (The oxidation of dithizone by the HNO_3 does not interfere with the transfer of Bi to the aqueous phase.) Drain and discard the $CHCl_3$ layer. Shake the third funnel for 10 second periods with successive 5 ml portions of $CHCl_3$ until both the aqueous and $CHCl_3$ layers are colorless. Discard the washes, but allow the final (colorless) wash to remain in the funnel.

The lead is now present in the aqueous phase in the second funnel and the bismuth in the aqueous phase in the third funnel. Determine lead by (1) or (2). Determine bismuth by (3).

(1) *Lead by electrolysis and titration with 0.001 N* $Na_2S_2O_3$.—Add 3 drops of thymol blue soln to the second funnel. Add NH_4OH dropwise to the appearance of a blue color. Add 2–3 ml of 10% KCN soln. Shake for 10 seconds. Drain and discard the $CHCl_3$. Extract the lead with a $CHCl_3$ soln of dithizone (50 mg per liter) and transfer the $CHCl_3$ layer to a dry funnel. Add exactly 110 ml of 1% HNO_3 and shake vigorously for 1 min. Discard the $CHCl_3$ and determine lead by electrolysis and titration with 0.001 *N* $Na_2S_2O_3$ as directed in **29.41**, *Methods of Analysis, Sixth Ed.*

(2) *Lead colorimetrically.*—Add exactly 10 ml of ammonia-cyanide reagent A to the second funnel. Shake for 10 seconds and remove the cover of the funnel to allow the $CHCl_3$ on the surface to evaporate (about 10 min). Drain and discard the $CHCl_3$, allowing the aqueous phase to fill the stopcock bore. Add exactly 15 ml of dithizone soln (15 mg per liter of $CHCl_3$) and shake vigorously for 1 min. Drain and discard 1–2 ml of the $CHCl_3$ phase. Dry the stem of the funnel with a cotton swab and insert a clean pledget of absorbent cotton. Determine the absorbency of the $CHCl_3$ phase at 510 mμ after discarding the first 3–4 ml which pass thru the cotton filter.

$$\text{Pb, mmg} = \frac{(A_{\text{sample}} - A_{\text{reagent blank}})\ 50}{A_{\text{standard}} - A_{\text{reagent blank}}}$$

where A represents the absorbency of the indicated solutions.

(3) *Bismuth colorimetrically.*—Add exactly 25 ml of ammonia-cyanide reagent B to the third funnel. Shake for 10 seconds and remove the cover of the funnel to allow the $CHCl_3$ on the surface to evaporate. Drain and discard the $CHCl_3$ allowing the aqueous phase to fill the stopcock bore. Add exactly 25 ml of dithizone soln (50 mg per liter of $CHCl_3$) and shake vigorously for 1 min. Drain and discard 1–2 ml of the $CHCl_3$ phase. Dry the stem of the funnel and insert a pledget of cotton. Determine the absorbency of the $CHCl_3$ layer at 490 mμ, after discarding the first 3–4 ml which passes thru the cotton filter.

$$\text{Bi, mmg} = \frac{(A_{\text{sample}} - A_{\text{reagent blank}})\ 100}{A_{\text{standard}} - A_{\text{reagent blank}}}$$

DISCUSSION

In the presence of cyanide only lead, bismuth, tin (stannous), and thallium (thallous) are extracted by dithizone. Clifford (1) has stated that stannic tin is not reduced by hydroxylamine under the conditions of the initial extraction. This was confirmed by adding tin to several samples. Within the limits of accuracy of the procedure, no interference was observed. The occurrence of thallium is considered unlikely.

The separation of lead and bismuth is carried out in the second funnel. The method is similar to previously published procedures in that it is based on the proper control of *p*H. Welcher (4) has summarized the vari-

ous methods proposed. The *p*H is readily adjusted to 2.5 ± 0.2 *p*H unit through the use of thymol blue indicator, as proved by measurements with a Beckman *p*H meter. The apparent *p*H is referred to in this discussion since the measurements were made in solutions saturated with chloroform. When a *p*H meter is available the analyst should satisfy himself as to the proper shade of color by similar measurements.

The *p*H is quite resistant to change through the action of chloroform. For example, 50 ml. of ammonium nitrate solution was adjusted to *p*H 2.5, and shaken with three successive 100 ml. portions of chloroform. The original and final *p*H as determined on a Beckman *p*H meter differed by less than 0.1 of a *p*H unit.

Lead is extracted at a *p*H of about 9.2 and bismuth at a relatively high *p*H of about 10.5 according to the recommendations of Hubbard (2). Because of the partitioning of dithizone between the aqueous and chloroform phases it is important that both the volume and *p*H of the aqueous phases in a given series of determinations be uniform. The ammonium nitrate, which is present in both the lead and bismuth determinations, has a pronounced stabilizing effect on the final *p*H, and no difficulty was experienced in this respect.

Laug (3) has shown that at a low *p*H, bromides form complexes with bismuth from which the metals cannot be extracted by dithizone. Iodides form the same type of complexes and are carried over in the chloroform to the second funnel where they could interfere with the bismuth separation. Therefore, the digestion procedure was modified to insure the complete removal of both bromine and iodine. Following the removal of the halogens, the solution is digested with nitric acid to re-oxidize any tin which may have been reduced by the hot hydroxylamine hydrochloride.

The procedure as given is applicable to lead in the range 0–60 mmg and to bismuth in the range 0–120 mmg. In a one gram sample this corresponds to 0–60 p.p.m. for lead and 0–120 p.p.m. for bismuth. In the case of lead the range 0–120 mmg was also tested, using 25 ml of dithizone solution for the final evaluation and 100 mmg of lead as a standard. A considerable variation in both range and sensitivity can be obtained by varying the volume and concentration of the dithizone solutions and the length of the cell in which optical measurements are made. In this work a one centimeter cell was used throughout. Absorbencies were determined with a neutral wedge photometer.

When the analyses cannot be completed in one day, the solutions should be allowed to remain overnight at an acid *p*H. The ammonia-cyanide mixtures should be added only just before the final evaluation of the metals is to be made.

Table 1 gives the results of a number of recoveries of both lead and bismuth using the colorimetric procedures. Duplicate samples of each dye were analyzed, known amounts of lead and bismuth (and tin, in certain

cases) being added to one of the samples in the Kjeldahl flask and carried through the procedure. The samples analyzed varied from 1–4 grams.

TABLE 1.—*Recovery of lead and bismuth*

DYE	DYE NO METALS ADDED			DYE + ADDED METALS					
	SAMPLE GRAMS	Pb FOUND	Bi FOUND	Pb FOUND	Pb ADDED	Pb RECOVERED	Bi FOUND	Bi ADDED	Bi RECOVERED
FD&C Red No. 2	1	0.8	0.0	39.5	40.0	38.7	102.1	100.0	102.1
FD&C Orange No. 1	1	0.0	0.0	50.0	50.0	50.0	98.9	100.0	98.9
FD&C Red No. 1	1	0.0	0.6	59.3	60.0	59.3	119.6	120.0	119.0
FD&C Yellow No. 5	1	3.2	0	23.3	20.0	20.1	77.0	80.0	77.0
D&C Red No. 7 Ca Lake	1	6.0	0.4	56.9	50.0	50.9	48.7	50.0	48.3
FD&C Red No. 3	1	2.4	0.5	51.6	50.0	49.2	62.2	60.0	61.7
D&C Orange No. 16	1	2.3	1.8	21.4	20.0	19.1	40.7	40.0	38.9
FD&C Blue No. 1	1	8.8	0.0	49.2	40.0	40.4	79.4	80.0	79.4
FD&C Red No. 3 Al. Lake	1	4.4	0.0	35.0	30.0	30.6	100.3	100.0	100.3
FD&C Red No. 3	4	1.4	0.0	102.1	100.0	100.7	39.4	40.0	39.4
D&C Red No. 21	2	0.0	0.0	119.7	120.0	119.7	20.0	20.0	20.0
D&C Red No. 11 Ca. Lake	2	32.5	0.2	92.9	60.0	60.4	60.7	60.0	60.5
FD&C Green No. 2	4	2.1	0.1	82.1	80.0	80.0	81.1	80.0	81.0
FD&C Green No. 2	4	3.0	0.0	82.9	80.0*	79.9	79.5	80.0	79.5
FD&C Yellow No. 4	4	1.8	0.0	63.4	60.0†	61.6	100.0	100.0	100.0
FD&C Red No. 3	1	29.4	1.3	90.0	60.0†	60.6	81.0	80.0	79.7

* 500 mmg Sn added.
† 1000 mmg Sn added.

The effectiveness of the procedure in separating larger quantities of bismuth (500 mmg.) was also investigated. In this case the bismuth was not determined. The lead was determined by electrolysis and titration with sodium thiosulfate. The results, expressed in terms of milliliters of approximately 0.001 *N* $Na_2S_2O_3$ solution, are shown in Table 2.

TABLE 2.—*Milliliters of approximately 0.001 N* $Na_2S_2O_3$ *solution*

	1	2	3	4	5	6	7	8	9	10	11
FD&C Yellow No. 6	0.12	0.95	0.12	0.96	0.95	0.99	0.09	0.90	0.83	0.84	0.83
FD&C Red No. 2	0.16	1.75*	0.24	1.05	1.07	1.89*	0.09	0.90	0.80	0.81	0.83
FD&C Yellow No. 6	0.09	0.94	0.12	0.96	0.95	0.97	0.07	0.90	0.85	0.84	0.85
FD&C Orange No. 1	0.11	0.90	0.25	1.06	1.07	0.97	0.07	0.90	0.79	0.81	0.82
FD&C Blue No. 2	0.17	1.77*	0.15	0.97	0.92	1.85*	0.08	0.89	0.80	0.82	0.77

* 200 mmg Pb added.

Column 1—Reagent blank.

Column 2—Reagent blank to which 100 mmg (or 200 mmg) of lead was added in the first funnel.

Column 3—4 grams of dye—no metals added.

Column 4—4 grams of dye plus 100 mmg of lead added to the Kjeldahl flask.

Column 5—4 grams of dye plus 100 mmg of lead plus 500 mmg of bismuth added to the Kjeldahl flask.

Column 6—100 mmg of lead added directly to 1% HNO_3 in beaker and electrolyzed.

Column 7—electrode blank.

The calculations are shown in columns 8–11. They are simply as follows:

Column 8 = column 6 minus column 7 (when 200 mmg of lead was added, the result was divided by two).

Column 9 = column 2 minus column 1 (when 200 mmg of lead was added, the result was divided by two).

Column 10 = column 4 minus column 3.

Column 11 = column 5 minus column 3.

The results in columns 8–11 represent the milliliters of approximately 0.001 *N* $Na_2S_2O_3$ required per 100 mmg of lead under the respective conditions. The general agreement of column 10 (dye plus no bismuth) with column 11 (dye plus bismuth) shows that at least 500 mmg of bismuth can be effectively separated.

SUMMARY

A method of separating and determining micro quantities of lead and bismuth in the analysis of coal-tar colors has been described. Lead is determined by electrolysis and titration or colorimetrically. Bismuth is determined colorimetrically. Lead in significant amounts occurs fairly frequently. Bismuth does not appear to be a serious contaminant.

RECOMMENDATIONS*

It is recommended that this procedure be submitted to collaborative study.

REFERENCES

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(4) WELCHER, F. J., Organic Analytical Reagents, Vol. 3., D. Van Nostrand Co., Inc., New York, N. Y.

REPORT ON HEAVY METALS IN COAL-TAR COLORS—COPPER

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Sodium diethyldithiocarbamate is a well-known reagent for the determination of micro-quantities of copper. A considerable number of publications describing its properties have appeared. References found useful in this work include those of Callan and Henderson (1), Haddock and Evers (3), Ovenston and Parker (5), and Welcher (6).

Greenleaf (2) has employed dithizone to isolate copper before the final determination with carbamate. The procedure described in this report follows the same general method. However, a number of modifications

* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951)

have been introduced which are believed to simplify the procedure materially. The modifications introduced reduce the false blank, obtained when Greenleaf's procedure is followed, to negligible proportions.

METHOD

REAGENTS

Ammonium chloride soln, 15% w/v.—Make the soln alkaline to litmus with NH_4OH. Remove heavy metals by shaking with a $CHCl_3$ soln of dithizone. Remove excess dithizone by shaking with successive portions of $CHCl_3$.

Triammonium citrate soln, 15% w/v.—Remove heavy metals as described above. A soln of citric acid (or lower ammonium salts of citric acid) made alkaline to litmus with NH_4OH can be used.

Ammonium thiocyanate soln, 15% w/v.—Remove heavy metals as before. Adjust the *p*H of the soln with HCl so that it is essentially neutral to litmus.

Potassium bromide soln, 40% w/v.—Remove heavy metals as described above.

Sodium diethyldithiocarbamate, 1% soln in water.—Prepare fresh daily. Filter before using.

Sodium hypochlorite, C.P.—Soln containing 5–6% available chlorine.

Copper standards.—Pure uneffloresced $CuSO_4 \cdot 5H_2O$ is satisfactory. Prepare a stock soln containing 1 mg of Cu per ml of soln. Prepare standards containing 10–20 mmg of Cu per ml by dilution of the stock soln.

Other reagents as under cadmium.*

PROCEDURE

Digest 1 g of dye and two reagent blanks as directed under cadmium.* Insure the complete removal of $HClO_4$ by continuing the digestion for 20 min. after the disappearance of the last trace of green color. Treat with citric acid, NH_4OH, and $NH_4OH \cdot HCl$, and transfer the soln to 250-ml separatory funnel as described under cadmium. Add 50 mmg of Cu to one of the blanks. This is the standard; the second blank is the reagent blank. Extract all dithizone metals by shaking for one-min. periods with successive 10-ml portions of dithizone soln (200 mg/liter in $CHCl_3$) and transfer the $CHCl_3$ layers quantitatively to a second 250–ml separatory funnel. Discard the contents of the first funnel.

Add 50 ml of 15% NH_4Cl soln to the second funnel. Add 3 drops of thymol blue soln. Add conc. HCl dropwise until the aqueous phase is red; then add several drops in excess. Shake the funnel for 10 seconds and add a drop of thymol blue soln. The aqueous phase should be red. If it is not, add several drops of HCl and treat as before. Add 5 drops of NaOCl soln and shake vigorously for 1 min. The dithizone and dithizonates are oxidized almost immediately as shown by the appearance of a deep orange color which changes to yellow upon continued shaking. The settled $CHCl_3$ phase should be yellow. If it is orange or red, add 5 drops of NaOCl and treat as before.

Drain and discard the $CHCl_3$ phase. Add 10 ml of $CHCl_3$ and 2 ml of $NH_2OH \cdot HCl$ soln and shake vigorously for 15 seconds. Drain and discard the $CHCl_3$. Add 5 ml of 40% KBr soln and 5 drops of thymol blue soln. If the indicator fades, NaOCl is present; add a little $NH_2OH \cdot HCl$ soln. Adjust the *p*H to 2.2 by adding NH_4OH dropwise, using dilute (1+20) NH_4OH for the final adjustment. The desired *p*H is indicated by a very light orange color. (Disregard the red color which appears in the residual $CHCl_3$.) Wash the contents of the funnel by shaking for 10-second periods with successive 10 ml portions of $CHCl_3$ until both phases are colorless. About three washes are required. Discard all of the $CHCl_3$ washes.

* *This Journal*, page 417.

Extract the Cu from the second funnel by shaking vigorously for 1-min. periods with successive 10-ml portions of dithizone soln (100 mg/liter of $CHCl_3$). Continue the extractions until 2 successive portions of dithizone show no color change (100 mmg of Cu can be removed with 4 extractions). Combine all of the dithizone extracts quantitatively in a third funnel. Wash the aqueous phase in the second funnel by shaking with 10 ml of $CHCl_3$ and add the washings to the third funnel. Discard the contents of the second funnel.

Add 2 ml of conc. HCl to the third funnel and shake vigorously for 30 seconds. Add 25 ml of 15% NH_4CNS soln and shake vigorously for one min. Drain and discard the $CHCl_3$ phase. Pass several small portions of $CHCl_3$ thru the funnel to remove the bulk of the remaining dithizone. Remove the last trace of dithizone by shaking with successive 5-ml portions of $CHCl_3$ until the $CHCl_3$ phase is colorless.

Add 25 ml of 15% ammonium citrate soln and several drops of thymol blue soln. Add conc. NH_4OH dropwise to the appearance of a blue color. Wash the aqueous phase with successive 5-ml portions of $CHCl_3$ until the $CHCl_3$ phase is completely colorless. (The first wash may be cloudy.) Discard all of the washes. Add 10 ml of $CHCl_3$ and shake the funnel for several seconds. Remove the cover of the funnel. Allow the surface layer of $CHCl_3$ to evaporate and the remainder of the $CHCl_3$ to settle (about 20 min.). Drain and discard the $CHCl_3$ layer allowing the aqueous layer to fill the stopcock bore.

Add 5 ml of carbamate reagent and exactly 10 ml of $CHCl_3$. Shake vigorously for one min. Allow 1–2 ml of the $CHCl_3$ layer to pass thru the bore of the stopcock. Dry the stem of the funnel with a clean swab of absorbent cotton and insert a fresh pledget of cotton.

Determine the absorbency at 440 mμ of the $CHCl_3$ layer after discarding the first 1–2 ml which pass thru the cotton filter.

$$\text{Cu, mmg} = \frac{(A_{\text{sample}} - A_{\text{reagent blank}})\ (50)}{A_{\text{standard}} - A_{\text{reagent blank}}}.$$

The addition of the carbamate reagent and all following operations should be carried out in the absence of direct sunlight. These operations, including the optical measurements, should be completed within one hour.

DISCUSSION

The method of digesting the dye and extracting all dithizone metals from an ammoniacal citrate solution is the same as that employed previously for cadmium. It is especially important to expel all of the perchloric acid.

In the second funnel the dithizone and metal dithizonates are oxidized with sodium hypochlorite, the metals reverting to the aqueous phase. Qualitative observations indicate that copper dithizonate is more readily oxidized than dithizone. However, the latter forms an intermediate red or orange product which may be confused with copper dithizonate. To avoid doubt on this point, the oxidation with sodium hypochlorite is repeated until the chloroform phase is yellow.

The yellow product resulting from the oxidation of dithizone is also soluble in the aqueous phase, but it does not interfere with the adjustment of the *p*H and is readily washed out by chloroform. The procedure requires a considerable number of chloroform washes but the chloroform and

aqueous phases separate quite rapidly and the analysis is not unduly long.

The copper is next extracted from the aqueous solution in the presence of bromides at a *p*H of about 2.2. Of the metals combining with dithizone, only nickel, cobalt, manganese, and bismuth, in addition to copper, are capable of forming colored compounds with the carbamate reagent used for the determination of copper. Nickel, cobalt, and manganese do not react with dithizone at a *p*H of about 2.2. Bismuth does not combine with dithizone at a low *p*H in the presence of bromides as shown by Laug (4).

The method employed to control and stabilize the *p*H is simple and reliable. In the case of the analyses shown in Table 1, the final *p*H of the solutions, after the copper had been removed, was determined with a Beckman *p*H meter. For the 64 measurements involved, the *p*H values ranged from 1.8 to 2.6, with a very great majority lying in the range of *p*H 2–2.4.

In the third funnel the copper dithizonate is decomposed by a small quantity of concentrated hydrochloric acid. The mixture cannot be treated with water at this point, because in the presence of dilute hydrochloric acid, dithizone will recombine with copper. However, when the mixture is shaken with a fairly strong solution of ammonium thiocyanate, at a low *p*H, the copper remains in the aqueous phase. The dithizone is removed before raising the *p*H, since at a higher *p*H copper will recombine with dithizone even in the presence of ammonium thiocyanate.

The scale of the neutral wedge photometer with which the optical measurements were made is graduated in millimeters. For the first ten analyses (Table 1) the blank readings ranged from 4.2 to 5.6 mm, the average being 4.9 mm. For the same ten analyses, the readings for the standard (50 mmg of copper) ranged from 52.0 mm to 53.0 mm, the average being 52.4 mm. The difference between the two averages (47.5 mm) represents the absorbency corresponding to 50 mmg of copper.

The blank appears to be due primarily to the presence of copper in the reagents used to digest the dye and to the leaching of copper from the glassware during the digestion. When a reagent blank was analyzed with the addition of potassium cyanide to the first funnel to prevent the extraction of any copper present, the blank reading was reduced to 1.0 mm. However, doubling the amount of reagents used failed to double the size of the blank, as would be expected if the blank were due entirely to copper present in the reagents. In any case, the blank appears to be quite uniform.

When many determinations are to be made, the inclusion of a standard with each series of analyses is unnecessary, once the absorbency corresponding to a given quantity of copper has been established. Thus, no serious error was found when analyses 1–10, inclusive (Table 1) were recalculated using the relationship: $\text{Cu(mmg)} = (A_{\text{sample}} \div 47.5)\ (50)$. The constant corresponding to a given quantity of copper depends, of course, on the cell, volume of chloroform, and instrument used. (An alternate method consists in the preparation of a standard curve.)

TABLE 1.—*Results of 16 analyses*

DYE	DYE PLUS NO ADDED METALS	DYE PLUS ADDED METALS			MMG OTHER METALS ADDED
	MMG Cu FOUND	MMG Cu FOUND	MMG Cu ADDED	MMG Cu RECOVERED	
1. FD&C Red No. 3	2.8	83.1	80.0	80.3	200 Co 200 Ni 200 Bi
2. FD&C Green No. 1	8.5	59.1	50.0	50.6	500 Bi 500 Mn
3. D&C Orange No. 4, Al Lake	12.2	52.6	40.0	40.4	1000 Bi
4. D&C Red No. 11, Ca Lake	16.7	42.3	25.0	25.6	1000 Co
5. Ext. D&C Green No. 1, ca 6% Fe	3.3	63.7	60.0	60.4	1000 Ni
6. D&C Red No. 28	0.5	74.3	72.0	73.8	300 Co 300 Ni 300 Bi
7. FD&C Yellow No. 3	2.7	14.4	12.0	11.7	None
8. FD&C Yellow No. 5	0.4	48.8	48.0	48.4	None
9. FD&C Blue No. 2	16.2	47.5	30.0	31.3	None
10. FD&C Red No. 4	8.7	85.2	75.0	76.5	None
11. FD&C Yellow No. 6	0.0	100.2	100.0	100.2	None
12. FD&C Orange No. 1	25.7	173.9	150.0	148.2	None
13. D&C Red No. 12, Ba Lake Blanc Fixe	24.3	71.6	50.0	47.3	None
14. D&C Red No. 30, Lake Talc	5.2	45.0	40.0	39.8	None
15. D&C Red No. 12, Ba Lake Blanc Fixe	10.5	74.2	65.0	63.7	None
16. D&C Red No. 9, Ba Lake Gloss White	40.6	68.0	30.0	27.4	None

Table 1 gives results obtained with 16 typical dyes and lakes. The indicated amounts of copper and other metals were added to 1 gram of dye in a Kjeldahl flask and carried through the entire procedure. With 10 ml of chloroform and a 1-centimeter cell, a range of 0–85 mmg of copper was covered. By using 20 ml of chloroform (and 100 mmg of copper in the standard) the range was readily increased to about 170 mmg. of copper (analyses 11 and 12).

In the case of barium lakes, there appears to be a slight loss of copper. Good results were obtained with a talc lake. The blanks obtained when filtration was necessary were approximately double the normal blank. This is believed to be due to the presence of copper in the filter paper.

SUMMARY

A procedure for the determination of copper in certifiable coal-tar colors has been described. Copper is isolated by means of dithizone and determined with sodium diethyldithiocarbamate.

RECOMMENDATIONS*

It is recommended that this procedure be submitted to collaborative study.

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REPORT ON HYGROSCOPIC PROPERTIES OF CERTIFIED DYES

By CHARLES STEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

No systematic study appears to have been made of the change in moisture content of certifiable coal-tar colors under varying conditions of temperature and relative humidity. Since the Coal-Tar Color Regulations (1) specify a maximum allowable volatile matter for each certifiable dye, this problem is of considerable importance. The results of a study of the hygroscopic properties of eighteen certifiable colors are given in this report.

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* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951).

APPARATUS

The literature contains many descriptions of apparatus designed to regulate humidity and temperature; such equipment is in general quite elaborate and costly. Solutions of salts, acids, and bases have long been employed to control humidity within small enclosed chambers. These methods have been reviewed by Buxton (2). Wilson (3) has summarized the advantages of the sulfuric acid method.

The controlled humidity apparatus assembled for this work consisted of a desiccator provided with stirring devices for both the sulfuric acid solution used to control the humidity and the air around the samples. The desiccator had a capacity of twenty (40×50 mm) weighing bottles. The desiccator lid was provided with a centrally located opening through which a mercury sealed glass stirrer (60 r.p.m.) was inserted. This stirrer extended into the humidifying mixture through an opening in the desiccator plate. In order to provide circulation of the air in the system, two fan-shaped metal blades were attached to the stirrer in such a manner that they rotated approximately 1 inch above the samples.

The desiccator was submerged to within about two inches from the top in a constant temperature (±1°C.) water bath. The inside of the desiccator above the desiccator plate and the desiccator lid were lined with heavy blotting paper as insulation.

One liter of sulfuric acid solution, prepared according to Wilson to give approximately the desired humidity, was placed in the desiccator well. Water alone was of course used in the 100 per cent humidity tests.

PROCEDURE

The dyes examined were commercial colors submitted for certification. One to five-gram samples were placed in weighing bottles. The moisture content was determined on separate samples by drying at 135°C. and the weight of dry dye in the test samples was calculated. The moisture percentages reported are based on the weight of dry dye.

Each dye was run in duplicate and two empty weighing bottles were carried as blanks. The bottles were weighed at least twice a week until successive weighings were reasonably constant. This required about three weeks at each relative humidity.

The hygroscopic properties of the dyes were determined at relative humidities of approximately 20, 40, 60, 80, and 100 per cent. When constant weight was established, the density of the acid was determined (pycnometer) and the relative humidity derived from the data compiled by Wilson (3).

RESULTS

Two groups of nine dyes each were examined at 25°C. and 35°C. The results are shown in Tables 1 and 2. Figures 1 and 2 give the plots of these

results at 25°C. Although the results obtained at 100 per cent humidity were too erratic to be of much significance, the water-soluble dyes did show a marked increase in moisture content, as would be expected. Fairly good agreement between duplicate samples was noted at relative humidities of 20–80 per cent. At higher relative humidities there was frequently considerable divergence. This may have been due to the "caking" of the dye observed to occur at the higher humidities.

TABLE 1

	PER CENT MOISTURE				PER CENT MOISTURE			
	25°C.				35°C.			
Relative humidity per cent	22.0	34.0	58.0	78.0	21.0	39.0	55.0	72.0
FD&C Blue No. 1	10.3	13.1	18.7	32.1	6.7	10.7	16.7	31.6
	10.3	13.1	18.7	32.1	6.7	10.6	16.8	31.9
FD&C Blue No. 2	8.1	9.0	10.3	29.9	6.8	7.8	9.1	28.9
	8.1	9.0	10.3	29.6	6.8	7.8	9.1	29.8
FD&C Green No. 1	8.1	10.3	14.0	21.6	5.7	8.1	12.3	23.1
	8.1	10.3	14.0	21.8	5.7	8.1	12.4	21.9
FD&C Green No. 2	10.1	12.8	17.8	30.0	6.7	10.0	15.6	29.4
	10.1	12.8	17.8	30.3	6.7	10.1	15.7	29.9
FD&C Green No. 3	10.4	12.1	16.2	24.6	7.3	9.4	12.4	23.0
	10.4	12.1	16.2	24.6	7.3	9.4	12.5	23.1
FD&C Red No. 1	11.9	14.3	17.6	24.8	8.8	12.6	16.0	25.2
	11.9	14.3	17.6	24.7	8.8	12.6	16.1	25.3
FD&C Red No. 2	8.1	8.6	9.3	27.4	6.6	7.5	8.2	27.3
	8.1	8.6	9.3	27.2	6.6	7.5	8.2	28.0
FD&C Red No. 3	12.8	15.3	17.4	19.4	11.4	13.7	16.5	18.8
	12.8	15.3	17.4	19.0	11.4	13.7	16.5	18.8
FD&C Orange No. 1	7.3	8.9	11.3	18.2	5.9	7.5	9.8	21.0
	7.3	8.9	11.3	18.2	5.9	7.5	9.8	20.5

The triphenylmethane dyes became liquids or glassy pastes at about 80 per cent relative humidity at both 25°C. and 35°C.

The absorption of water by the colors examined does not appear to be a completely reversible phenomenon under the experimental conditions employed. After the dyes shown in Table 1 were allowed to reach equilibrium at relative humidities of approximately 20, 40, and 60 per cent (in that order), the samples were placed in an atmosphere of approximately 40 per cent relative humidity and later at 20 per cent.

TABLE 2

	PER CENT MOISTURE				PER CENT MOISTURE			
	25°C.				35°C.			
Relative humidity per cent	19.5	35.0	55.0	79.0	11.5	31.0	55.0	77.0
FD&C Red No. 4	3.8	5.4	5.7	19.1	3.7	5.2	5.6	21.6
	3.8	5.4	5.7	20.0	3.7	5.2	5.7	20.2
FD&C Yellow No. 1	7.4	7.4	7.4	17.0	7.3	7.3	7.4	17.4
	7.4	7.4	7.4	17.0	7.3	7.3	7.4	17.5
FD&C Yellow No. 5	6.6	12.5	20.5	45.9	5.8	11.3	20.2	38.8
	6.6	12.5	20.5	44.5	5.8	11.3	20.2	38.7
FD&C Yellow No. 6	7.9	10.3	15.4	32.7	7.3	9.4	16.1	33.4
	7.9	10.4	15.4	32.7	7.1	9.2	15.7	33.1
D&C Red No. 18	1.0	1.0	1.1	1.4	1.8	1.8	1.9	2.3
	1.0	1.0	1.0	1.4	1.9	1.8	2.0	2.4
D&C Red No. 19 (Al Lake)	3.7	4.3	5.1	7.3	3.5	3.9	4.7	6.8
	3.6	4.3	5.1	7.3	3.5	4.0	4.8	6.9
D&C Red No. 21	0.1	0.2	0.2	0.4	0.2	0.2	0.3	0.5
	0.1	0.2	0.2	0.4	0.2	0.2	0.3	0.5
D&C Red No. 22	5.3	9.3	14.0	22.8	5.0	8.0	13.3	22.3
	5.2	9.3	14.0	22.8	5.0	8.0	13.3	22.4
D&C Red No. 31	1.0	1.3	1.7	6.8	0.8	1.1	1.5	6.8
	1.0	1.2	1.7	6.7	0.9	1.1	1.4	6.8

It was found that the samples reached equilibrium at a higher moisture content after exposure at 60 per cent relative humidity. The difference was about 2 per cent.

CONCLUSIONS

The hygroscopic properties of dyes appear to be entirely consistent with their known solubilities in water. Thus the water-soluble dyes readily absorb water, whereas the water-insoluble dyes do not. Temperature, within the range investigated, has little effect on the water-absorbing capacity of dyes.

RECOMMENDATIONS*

In view of the normal behavior of the eighteen dyes examined, it is recommended that further work on this subject be discontinued.

* For report of Subcommittee B and action of the Association, see *This Journal*, 34, 46 (1951).

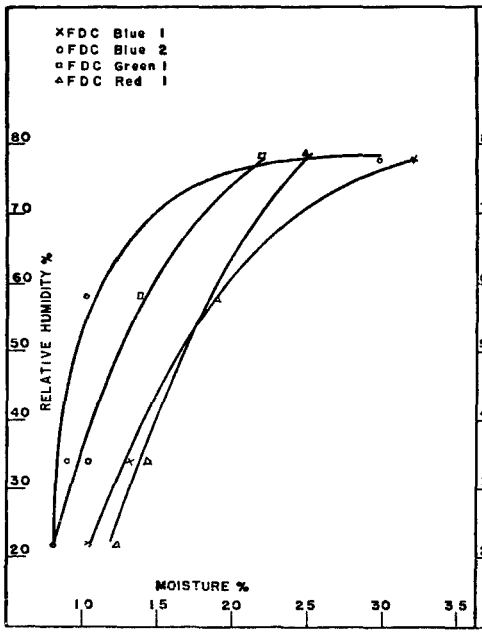

FIG. 1.

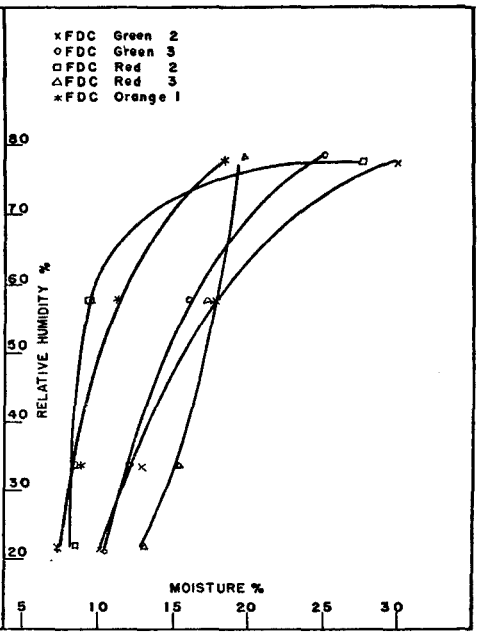

FIG. 2.

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Three contributed papers by Lee S. Harrow, entitled "Determination of Uncombined Alpha- and Beta-Naphthol in Coal-Tar Colors," "Determination of β-Naphthylamine in FD&C Yellow Nos. 3 & 4," and "Determination of *m*-Diethylaminophenol in D&C Red No. 18 and D&C Red No. 37," were published in the preceding number of *This Journal*, **33**, (1951) pages 127, 131, and 133, respectively.

REPORT ON SUGARS AND SUGAR PRODUCTS

By CARL F. SNYDER (National Bureau of Standards, Washington 25, D.C.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That the study of methods for the determination of moisture be continued.

(2) That the study be continued on tables of density of solutions of sugar at various temperatures.

(3) That the study of methods for the detection of adulteration in honey be continued.

(4) That methods for the determination of reducing sugars, including those employing chromatographic separation, be studied.

(5) That the Zerban and Martin values for the refractive indices of dextrose and of invert sugar solutions be adopted as official (*This Journal*, **27**, 295 (1944)).

(6) That the tentative methods, **29.132–29.154,** inclusive, adopted as first action last year, be further studied.

(7) That the procedure for the measurement of transmittancy of solutions of commercial sugar products be further studied.

(8) That the micro method for dextrose, **29.61–29.63,** be adopted as official.

REPORT ON REDUCING SUGARS

By EMMA J. MCDONALD (National Bureau of Standards, Washington, D.C.), *Associate Referee*

The food chemist is frequently required to analyze products containing sugar mixtures. Whereas the determination of a single sugar is a comparatively simple procedure, when two or more reducing sugars are present the difficulty of analysis is greatly increased. Starch conversion products or corn sirups containing dextrose, maltose, and dextrins are no exception. The dextrose equivalent is frequently used to characterize these products; as the name implies this is merely expressing the reducing power of the sirup in terms of the reducing power of dextrose. Lane and Eynons' method is the procedure used by the Corn Industries for this analysis, although Munson and Walker's method gives comparable results. Selective chemical and enzymatic methods have been used for a more complete analysis of these products.

Zerban (1), when determining dextrose and maltose in three sirups, representing low, medium, and high starch conversion products, found con-

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 56 (1951).

siderable discrepancies in the results obtained by use of different methods, especially in the case of maltose. Discarding the results obtained by a procedure in which the dextrins are included in the maltose fraction, the maltose found varied from 6.4 to 11.2 per cent, from 12.9 to 20.6 per cent, and from 15.5 to 22.3 per cent. The dextrose varied from 7.4 to 9.5 per cent, from 14.7 to 17.2 per cent, and from 28.8 to 30.1 per cent, in the respective sirups. Zerban concludes that "fermentation with specific organisms or perhaps chromatography might have to be resorted to."

It has long been recognized by the Referees of this Association that a method of sugar analysis, whereby the constituents of a sugar mixture could be determined, was greatly needed. We believe that an adaptation of a chromatographic procedure may well solve the problem.

A volume of chromatographic work has been described in the literature during the past few years and sugar mixtures have been included in the studies. Tiselius (2) in 1943 described the separation of glucose from sucrose, and sucrose from maltose, by use of charcoal columns on which 0.5 per cent phenol was used as the developing agent. Claesson (3) resolved mixtures of glucose, sucrose, and raffinose by use of 4 per cent aqueous phenol on similar columns. Montgomery (4), Weakley, and Hilbert used much the same technique in separating isomaltose from the enzymatic hydrolysis products of starch. They found that at *p*H 7 to 8 all sugars were adsorbed on the column, but that glucose could be removed by washing the column with water, while at *p*H 5.5 the glucose was adsorbed only weakly and soon was removed from the column. Whistler and Durso (5) found that a separation of monosaccharides, disaccharides, and trisaccharides could be accomplished on Darco-Celite columns using water and water-alcohol mixtures as developing agents. This appears to be a promising method of approach for an analytical procedure for starch hydrolysis products and work is now in progress at the National Bureau of Standards to refine such a procedure so that it may be used as a quantitative method adaptable to routine analysis.

Paper chromatography has received much attention by sugar chemists especially in Great Britain. De Whalley (6) and his associates have found that the identification of unfermentable reducing substances formed from fructose could be accomplished by a paper chromatographic method. He has also used the same technique for the determination of raffinose in raw sugars. Zerban and Sattler have successfully used this method for the identification of sugars in mixtures. Paper chromatography has also been adapted to macro analysis; in this procedure piles of filter paper or columns of cellulose are used.

From our experience it may be said that the chromatographic methods require careful detail attention but that they are most promising as a tool for separating sugar mixtures into components that can be analyzed by standard chemical methods. We realize that many laboratories are

experimenting along these lines and we welcome any comments you would care to make concerning these methods or other reducing sugar methods.

It is recommended* that methods for reducing sugars, including those employing chromatographic separations, be studied.

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Reports were not given for drying methods or for densimetric and refractometric methods, in sugars and sugar products.

The contributed paper entitled "Refractive Indices of Maltose Solutions," by F. W. Zerban and James Martin, was published in the preceding number of *This Journal*, **34**, 207 (1951), together with a paper under the same title by Emma McDonald.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

In the Referee's report last year it was recommended that the methods for pectic acid in the various forms of cacao products tested collaboratively last year be adopted as first action. The methods will be published in the new edition of *Methods of Analysis, A.O.A.C.* No work on pectic acid has been done this year.

The method for fat determination in refractory cacao products, proposed by the Associate Refreee last year, has been further tested collaboratively this year with respect to the use of larger samples (20 g) in the case of milk chocolate. The agreement between collaborators was excellent. The method was further tested by the Associate Referee on several refractory types of cacao products, compared with other methods, and found to give good results. This work is therefore completed and it is recommended* that it be closed.

The Associate Referee on Maltose has submitted a progress report suggesting a method for the determination of this sugar in mixtures with

* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 56 (1951).

dextrose, sucrose, lactose, and dextrin. The method proposes to obtain the maltose by determining the total reducing power of the sample, obtaining dextrose by the Steinhoff method[1] and lactose by copper reduction after fermentation, and then subtracting the reducing values of these sugars from the total reducing power of the sample to obtain the lactose. This method of attack appears as sound as any, in the opinion of the Referee, inasmuch as there is no specific method for maltose. The work should be continued along this line until such time as a more specific method for maltose may appear in the literature.

Some work has been done this year on the separation of cacao constituents, particularly tannins and theobromine, by ion exchange, but this work is not yet sufficiently advanced to make a report.

There is no report on lactose this year.

RECOMMENDATIONS*

It is recommended—

(1) That work on pectic acid be continued.

(2) That the modified acid hydrolysis method for fat in refractory cacoa products and milk chocolate, *This Journal*, **28**, 482 (1945), as further modified in the 1949 report of the Associate Referee, *This Journal*, **33**, 61 (1950), be made official and that the project be closed.

(3) That work on methods for determination of maltose in cacao products be continued.

(4) That work be continued on methods for the determination of lactose in cacao products containing dextrose, malt, or corn sirup solids.

(5) That work be continued on methods for the determination of cacao constituents, particularly theobromine, cacao red, and tannins.

(6) That the method for lecithin, *This Journal*, **32**, 168 (1949), be studied further.

REPORT ON MALTOSE IN CACAO PRODUCTS

By E. W. MEYERS (Chief Chemist, Hershey Chocolate Corporation, Hershey, Pa.), *Associate Referee*

This is a progress report on the determination of maltose in the presence of sucrose, lactose, dextrose, and dextrin. The project was started by the writer several years ago and more recently Peter Birnstiel and Karl Walter have continued this study.

From the beginning, a specific test for maltose has been sought but being unable to develop such a method, we have attempted to determine the other sugars by some specific means and then find the maltose by difference. To investigate the available methods and to determine the

[1] *Official Methods of Analysis, A.O.A.C.* 7th Ed. 29.148–29.150.

* For report of Subcommittee D and action of the Association, see *This Journal*, 34, 53 (1951).

most reliable one for each of the other sugars required considerable time. This procedure appears to be the one most likely to give the desired results.

Research was extended to fermentation methods using yeasts and molds and to chromatographic studies, especially the latter method; but as yet we have not found suitable conditions; *i.e.*, adsorbent, solvent, and indicator.

Assuming that it will be necessary to first determine each of the other sugars, it is recommended that the following method be used:

(1) *Sucrose*—Polariscope (Clerget Method).

(2) *Lactose*—Fermentation Method of Magraw, Copeland, and Sievert. This method has been used in our laboratory for several years and has given us excellent results.

(3) *Dextrose*—Steinhoff Method.

(4) *Dextrin*—Copper reduction before and after complete hydrolysis.

(5) *Maltose*—By difference, using one of several methods:

(A) *Polarization.*—After determining each of the other sugars, calculate their polarization; and the difference between this value and that obtained on the sample would equal the maltose.

(B) *Copper Reduction.*—The difference between the value found for the total reducing sugars and that found for the lactose and dextrose should equal the maltose. This value must be calculated as demonstrated in "Analysis of Sugar Mixtures" by Zerban and Sattler (*Ind. Eng. Chem.*, December 15, 1938, p. 669–674).

(C) *Refractometer.*—We are still studying the use of this instrument as a means for determining total carbohydrates.

It is planned to prepare special samples to send to collaborators to check results using the above method. In the meantime, it is proposed to continue investigations as follows:

(1) Specific fermentation method for maltose, or other combinations of fermentation methods.

(2) Determination of total carbohydrates by use of alkyd resins.

(3) Chromatographic analyses.

No report was given on cacao ingredients.

REPORT ON FAT IN MILK CHOCOLATE AND COCOA MIXES

By CARL B. STONE (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Last year Committee D recommended that a 10–20-gram sample be used in extracting the fat from milk chocolate or similar products. Use of the larger sample is advantageous when constants are to be run on the extracted fat. With this larger sample the same procedure as outlined for the Modified Hillig method, *This Journal*, **28**, 482 (1945), was followed as written. The average figure secured by ten analysts on the milk chocolate last year was 31.45% fat. Using this same sample and a 20-gram portion two analysts secured the following results:

Analyst	*Per cent fat*
I	31.52
	31.55
II	31.49
	31.46
	31.42
	31.41
	31.32
Average	31.45

The above results show that the percentage of fat recovered is not affected by the use of the larger sample with no increase of any of the chemicals as given in the method.

The Referee suggested that a few Reichert-Meissl determinations be run on the extracted fat samples so as to determine if any material is lost in the Modified Hillig method as given.

Several different methods were used in extracting the fat where there would be no chance of loss of the fat constituents as a comparison with the Hillig method. A 20-gram sample of milk chocolate was hydrolyzed with hydrochloric acid and then transferred to a Palkin extractor and the fat was removed by using petroleum ether as a solvent. Another set of 20-gram samples were hydrolyzed with hydrochloric acid, cooled and transferred to nursing bottles with 50 ml. portions of petroleum ether added. The bottles were stoppered and shaken very vigorously and then placed in a centrifuge for 15 minutes to settle the insoluble material. The ether was then decanted in tared beakers so as to record the recovery. Sufficient portions of ether were added to insure complete removal of the fat. The ether was evaporated and the Reichert-Meissl determination run on the fat. Several 20-gram samples were run by the Modified Hillig method so as to check the fat constants by the Reichert-Meissl method. The Reichert-Meissl results secured were calculated to the percentage

of butterfat in the milk chocolate sample being used in the three procedures outlined above. The results of the analyses are reported below:

	Per cent butterfat
Palkin Extraction Method	8.52
Centrifuge Procedure	8.73
	8.64
Modified Hillig Method	8.81
	8.87
	8.58
	8.91

The recovery of butterfat by the different procedures agree very closely. From these results it does not seem as if any of the fat constituents are lost in using the Modified Hillig method.

The General Refreee suggested that the Modified Hillig method be applied to other types of refractory samples such as cocoa mixes. Several of these products were secured and examined by the Modified Hillig method and a Modified Roese-Gottlieb method for fat in refractory cacao products. The results secured on the different products are reported below:

	Roese-Gottlieb method	*Modified Hillig method*
4—I Sweet Cocoa Mix	4.83%	4.82%
	4.60%	4.81%
	4.87%	
	4.96%	
Hot Chocolate Powder	14.43%	14.42%
	14.36%	14.58%
Non-Settling Powder	11.69%	11.29%
	11.77%	11.21%

The results secured on the three samples by the two methods agree very well with the exception of the non-settling powder, which shows a higher recovery by the Roese-Gottlieb procedure. In correspondence with the manufacturer of this product the higher figures were accounted for by the fact that a stabilizer was used and this was partly soluble in the alcohol treatment. The results by the Modified Hillig method are very close to the manufacturers formula in each case. This additional work shows that the Modified Hillig method gives good results on the various cocoa mixes.

ACKNOWLEDGMENT

The Associate Referee gratefully acknowledges the assistance of Frank Collins and F. J. McNall, Cincinnati District, for the analytical work performed.

RECOMMENDATIONS*

It is recommended—

(1) That a 20-gram sample of milk chocolate be used when the fat constants are to be determined.

(2) That the subject be closed.

REPORT ON FRUITS AND FRUIT PRODUCTS

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

During the past year consideration was given to methods for the separation and determination of citric and malic acids. Hartmann, *This Journal*, **26**, 456 (1943), published a revised pentabromacetone procedure for citric acid which makes it unnecessary to correct for solubility. This procedure with some editorial changes was subjected to collaborative study by Associate Referee L. W. Ferris. It appears that the revised procedure for citric acid is satisfactory and is to be preferred to the present first action procedure. The Referee concurs in the recommendation that the revised procedure be made official and that it replace the present first action procedure.

J. B. Wilson, *This Journal*, **33**, 995 (1950), obtained good recoveries of malic acid in synthetic solutions using ion-exchange resins for separation of the acid, followed by determination of the malic acid by the A.O.A.C. polarization procedure. This approach should be studied further to determine whether the procedure can be applied to fruits and fruit products.

The Referee is recommending a change under Preparation of Sample, **26.2(a)** juices. The use of muslin for filtering fruit juices has been in effect since the U. S. Dept. of Agriculture Bureau of Chemistry Bulletin No. 107, 77 (1908). Muslin has a variable identity and quality and it is no longer in general use in the chemical laboratory. Suspended matter in fruit juices can be more readily removed by filtration with conventional materials such as absorbent cotton or one of several types of filter paper.

No report was made by the Associate Referee for Fruit and Sugar in Frozen Fruits. Work on methods for the examination of frozen fruits and fruit juices should continue under the general title "Methods for the Examination of Frozen Fruits and Fruit Products."

RECOMMENDATIONS†

It is recommended—

(1) That the revised method for citric acid be adopted as official, in place of the present tentative method.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 53 (1951).
† For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 55 (1951).

(2) That work on methods for the separation and determination of fruit acids be continued.

(3) That **26.2**—Preparation of Sample (a) juices, be changed to read:

20.2 (a) Juices. Mix thoroly by shaking to insure uniformity in sampling, and filter thru absorbent cotton or rapid filter paper. Prep. fresh juices by pressing well-pulped fruit and filtering. Express juice of citrus fruits by one of the common devices used for squeezing oranges or lemons, and filter.

(4) That work on methods for the examination of frozen fruits and fruit products be continued.

REPORT ON CITRIC ACID IN FRUIT AND FRUIT PRODUCTS

By L. W. FERRIS (Food and Drug Administration, Federal Security Agency, Buffalo, N. Y.), *Associate Referee*

At the suggestion of the Referee on Fruits and Fruit Products, three samples were prepared and citric acid determined collaboratively by the method of Hartmann.[1] Since some changes in the wording of the method were suggested, the complete procedure was forwarded to each collaborator. The details of the method have been given in *This Journal*, **34**, 74 (1951).

Samples numbered 1 and 2 consisted of juice pressed from McIntosh apples to which was added 1 gram citric acid crystals per 100 ml. Collaborators were instructed to use 25 ml of sample number 1, which was in a large bottle, and 10 ml of sample number 2, in a small bottle, without being informed that the two samples were identical. Sample number 3 consisted of grape juice to which was added 0.5 gram of citric acid crystals per 100 ml. Collaborators were instructed to take for analysis a 25 ml aliquot of sample number 3. The fruit juices before and after the addition of citric acid were carried through the same procedure by the Associate Referee. Results on the apple juice showed 0.01 and on the grape juice 0.02 gram per 100 ml calculated as anhydrous citric acid. Results on the citric acid were 91.4, 91.9, and 92.1 per cent anhydrous acid (average 91.8)—Theoretical 91.4 per cent. The following table shows the results by the collaborators on the three samples:

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[1] *This Journal*, **26**, 456 (1943).

TABLE 1.—*Anhydrous citric acid in collaborative fruit juice samples expressed as grams per 100 ml*

SAMPLE NUMBER	1	2	3
Theoretical	0.92	0.92	0.48
Analyst 1	0.91	0.91	0.47
	0.91	0.89	0.47
2	0.93	0.96	0.49
	0.93	0.96	0.50
3	0.91	0.90	0.48
	0.92	0.90	0.48
4 Run I*	0.84	0.86	0.45
	0.88	0.87	0.46
Run II*	0.88	0.83	0.47
	0.88	0.83	0.46
5	0.97	0.99	0.46
	0.98	0.99	0.47
6	0.91	0.90	0.47
	0.91	0.91	0.47
	0.92	0.89	0.46
	0.92	—	0.47
7	0.92	0.89	0.47
	0.91	0.89	0.47
8	0.92	0.91	0.48
	0.92	0.91	0.47
Average	0.91	0.90	0.47
Maximum deviation from theoretical	0.08	0.09	0.03

* See under Comments of Collaborator 4.

EXCERPTS OF COMMENTS OF COLLABORATORS

3. In filtering the solution saturated with H_2S gas, it was found impossible to obtain a completely bright filtrate. The first portion of filtrate would be bright but by the time filtration was completed, a haziness had developed. Therefore, the solutions were centrifuged before taking the required aliquots.

4. Before undertaking this work the proposed method was compared with the published methods, *This Journal*, **26**, 456 (1943), and *Book of Methods*, **26.37**. The latter methods prescribed crystallization "in refrigerator overnight." Therefore, it was concluded for Run I that sodium sulfate could be added to all determinations at the same time and after shaking these could be held at 10–15°C. for an hour, until

ready for filtration, then warmed to 15°C. The result was a large quantity of Na_2SO_4 crystals which did not redissolve on shaking at 15°C. even when a little excess water was added. The crystals were therefore filtered off and were nearly completely washed from the gooch crucibles with 50 ml of "cold water" at 15°C.

On Run II the loose interpretation of the word "immediately" was changed. Sodium sulfate was added at 15°C., shaken for 5 minutes; additional water in 2 ml portions was added, when necessary, to be sure that nearly all Na_2SO_4 had dissolved; cooled approximately 15 minutes at 15°C., then filtered. Finally the crucibles were washed with water at 3°C. Run II gave reasonably good duplicate checks on all samples whereas only Sample 2 gave good checks on Run I. The results on Sample 2 for Run I average 6 mg of pentabromoacetone higher than Run II. I do not have any explanation except that undercooling may have precipitated more PBA or the Na_2SO_4 in the crucible may have been dehydrated on the final drying.

A blank and control sample were carried through with Run I. The blank was negligible (<.0002 gm PBA) and the control of 0.40 gm anhydrous citric acid by titration gave a recovery of 98%.

5. The directions were clear and easy to follow, and the analysis proceeded smoothly with but two exceptions which are listed below:

1. While shaking the centrifuge bottle, after saturating the solution with H_2S, quite a high pressure was developed which was sufficient to blow out the stoppers in spite of their being held firmly in place. If the bottle was opened once or twice during the shaking, no trouble occurred.

2. On addition of the 20 g anhydrous Na_2SO_4 the salt caked unless the flask was shaken during the operation.

6. Removal of Pectin—procedure satisfactory.

Isolation of Polybasic Acids—

1. I was unable to find instructions for preparation of "finely powdered lead acetate." Normal lead acetate was dehydrated by heating in a 75–80°C. water bath in partial vacuum with small amount of air bled into flask. The anhydrous product was then powdered in porcelain mortar and stored in desiccator. Was this procedure and the resulting powdered lead acetate proper for the purpose? It seemed to work satisfactorily.

2. The final rinse with 80% alcohol produced a slight turbidity that centrifuging did not clear in the case of Sample #2 (2 of the 3 runs), but do not believe it was significant in amount.

Citric Acid (Normal)—

1. After addition of 5% $KMnO_4$ should sample be returned to 50° bath, or permitted to stand at room temperature for 5 minutes?

2. After addition of Na_2SO_4 and re-cooling to 15°C., should the sample be maintained at 15°C. during the 5 minutes shaking, or permitted to warm up as it naturally would during that period of shaking at room temperature (24°C.)? No difficulty was encountered with any of the precipitations or filtrations.

1. The method gives reproducible results and should be easily adapted to control work.

DISCUSSION

Several points have been brought up by the comments of collaborators.

(a) Holding temperature of solution after addition of $KMnO_4$. This does not seem to be critical, whether the solution is held at 50° for the 5 minutes or allowed to stand on the bench at room temperature.

(b) Dissolving the anhydrous sodium sulfate after removal of excess bromine.

It is undesirable to have undissolved sodium sulfate present when the pentabromacetone is filtered. Therefore, the salt should be completely in solution before the mixture is cooled to 15°C. The writer has found it convenient to carry only one determination at a time from the addition of the sodium sulfate to filtration and washing, so that the filtering can be done *immediately* after the 5 minutes shaking.

(c) Temperature of the water used to wash the pentabromacetone. This does not seem to be critical and any temperature between 0° and 15°C. should be satisfactory.

Appreciation is expressed to the following collaborators for their contributions: G. E. Palmer, American Can Co., Maywood, Ill.; and members of the Food and Drug Administration, Federal Security Agency: J. B. Wilson and J. Marder, Washington, D. C.; H. P. Eiduson, Buffalo, N. Y.; H. M. Bollinger, Los Angeles, Calif.; T. E. Strange, Portland, Oregon; T. J. Klayder, Denver, Colo.

RECOMMENDATIONS*

It is recommended that the method for citric acid as given in this report be made official.

No report was given on fruit and sugar in frozen fruit, or on volatile acids.

The contributed paper entitled "Use of Ion Exchange in the Determination of Fruit Acids," by J. B. Wilson, was published in *This Journal*, **33**, 995 (1950).

REPORT ON WATERS AND SALT

By C. G. HATMAKER (U. S. Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Since the extensive changes in last year's report, the Referee has begun a preliminary study on first action titrimetric method of determining boron in water, *This Journal*, **33**, 355 (1950). Results so far obtained on various samples of mineral waters indicate that the method will give reproducible results and good recovery when known amounts of boron are added. Collaborative study on this method is planned for the coming year.

There appears to be a need for a more accurate method for small quantities of phosphates in water. The method now appearing in this chapter, **31.33,** is well suited for phosphates in water samples when present in quantities above 4 mg. Samples containing less than 1 mg per liter require the time-consuming evaporation of a large quantity of water. A quicker

* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 55 (1951).

colorimetric procedure which could be applied directly on the sample would be of considerable advantage.

RECOMMENDATIONS*

It is recommended—

(1) That the first action method for boron in water be studied collaboratively.

(2) That a colorimetric method for phosphates in water be studied.

(3) That further study on methods for fluorine in salt be dropped.

ANNOUNCEMENTS

Referee Assignments, Changes, and Appointments

Economic Poisons:

E. L. Gooden, Bureau of Entomology and Plant Quarantine, Beltsville, Md., has been appointed as Associate Referee on Test Methods for Economic Poisons. Irwin Hornstein, of the same Bureau, has been appointed in place of C. V. Bowen, as Associate Referee on Benzenehexachloride.

CORRECTIONS IN NOVEMBER, 1950, JOURNAL

Page 997, par. 3, line 3, change "cation" to "anion"; line 4, change "anion" to "cation."

CORRECTIONS IN FEBRUARY JOURNAL

Page 46, par. (1) under "Coal-tar Colors," line 3, change "17" to "16."
Page 91, par. (1) under "34. Coloring Matters," line 3, change "17" to "16."
Page 61, line 3, par. (2), under 9. Beverages: Distilled Liquors, delete "(details given on p. 330)."
Page 77, line 23, reference **21.11**, change to **34.11**.
Page 85, under "Apparatus—Partition tube," line 1, delete words "3.85×200 mm test tube of."

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* For report of Subcommittee D and action of the Association, see *This Journal*, **34**, 57 (1951).

CONTRIBUTED PAPERS

UREA AND AMMONIACAL NITROGEN IN PACKING HOUSE FEEDING STUFF PRODUCTS[1]

By ALFRED T. PERKINS, J. F. MERRILL, and S. N. ROGERS (Department of Chemistry, Kansas State College, Manhattan, Kans.)

Recently the sale of urea for incorporation in livestock feeds has been increasing and it is desirable to check the amount of such additions. Urea is acceptable in feeds for ruminants only, and even for them large amounts are considered toxic. Normal packing house products contain small amounts of nitrogen that are recovered by the A.O.A.C. method for urea and ammoniacal nitrogen. The principal purpose of the present paper is to determine the amount of such nitrogen normally present in such feeding stuffs, so that the amount of added urea can be determined more accurately.

In undertaking the survey it was discovered that the A.O.A.C. method failed to give accurate recoveries when known amounts of urea were added to certain samples of packing house products. This was found to be due to the non-use of a buffer to control the *p*H of the solution during the urease digestion. This omission resulted in the failure of the urease to decompose the urea in certain samples that yielded suspensions either too basic or acidic in reaction.

The following method, patterned after the A.O.A.C. (1) method and that of Koch (2) was used to obtain the urea data herein presented:

One gram of sample is weighed into a Kjeldahl flask known to be free of heavy metals; 0.2 gm of urease, 300 ml of water and 1 ml of buffer solution are added. The contents of the flask are thoroughly mixed, tightly stoppered, and allowed to stand overnight at room temperature. An excess of MgO is then added with a bit of paraffin and ammonia is distilled as in a regular Kjeldahl determination for nitrogen. The receiving flasks are boiled to remove carbon dioxide, cooled and titrated. The buffer solution consisted of 140 gm of $Na_4P_2O_7 \cdot 10H_2O$ and 12 ml of 85% H_3PO_4 made up to a volume of one liter.

The method used for ammoniacal nitrogen only, is the same as the A.O.A.C. method for fertilizers and is as follows: One gram of sample is mixed with 300 ml of H_2O in a Kjeldahl flask; an excess of MgO is added and a bit of paraffin. The liberated ammonia is determined as in a regular Kjeldahl determination for nitrogen.

Most packing house products contain not only traces of ammoniacal nitrogen but also some urea. The data in Table 1 give the total nitrogen of 47 such samples as determined by the A.O.A.C. method for protein

[1] Contribution No. 446, Department of Chemistry, Kansas Agricultural Experiment Station, in cooperation with the Kansas State Board of Agriculture.

TABLE 1.—*Nitrogen content of packing house products*

LAB. NO.	TOTAL NITROGEN*	UREA AND AMMONIACAL NITROGEN*	AMMONIACAL NITROGEN*	DESCRIPTION
	per cent	*per cent*	*per cent*	
27002	8.39	0.08	0.08	Meat, Bone, & Hoof Scraps
27025	8.52	0.11	0.08	Meat, Bone, & Hoof Scraps
27026	8.54	0.13	0.12	Meat Scraps
27027	7.36	0.05	0.04	Meat & Bone Scraps
27028	8.63	0.14	0.14	Meat & Bone Scraps
27047	9.00	0.05	0.04	Meat & Bone Scraps
27063	—	0.08	0.03	Steamed Bone Meal
27064	8.02	0.09	0.07	Meat & Bone Scraps
27065	9.53	0.19	0.18	Digester Tankage
27069	8.39	0.10	0.09	Meat & Bone Scraps
27111	8.87	0.08	0.06	Meat & Bone Scraps
27124	9.39	0.08	0.07	Meat & Bone Scraps
27154	8.03	0.07	0.07	Meat & Bone Scraps plus limestone
27155	9.29	0.08	0.07	Meat Scraps
27173	8.06	0.06	0.05	Meat & Bone Scraps
27185	8.40	0.13	0.13	Meat & Bone Scraps
27222	8.50	0.07	0.05	Meat & Bone Scraps
27223	8.75	0.09	0.08	Meat Scraps
27229	8.89	0.09	0.07	Meat & Bone Scraps
27233	—	0.06	0.03	Steamed Bone Meal
27264	9.00	0.13	0.07	Tankage
27268	8.51	0.15	0.14	Meat Scraps
27287	7.89	0.06	0.05	Meat & Bone Scraps
27294	8.19	0.09	0.06	Meat Scraps
27327	—	0.08	0.06	Steamed Bone Meal
28344	8.22	0.12	0.10	Meat & Bone Scraps
27345	—	0.06	0.05	Steamed Bone Meal
27364	8.39	0.10	0.09	Meat & Bone Scraps
27368	8.03	0.08	0.06	Meat & Bone Scraps
27383	8.41	0.07	0.04	Meat & Bone Scraps plus limestone
27284	9.84	0.11	0.07	Tankage
27396	9.68	0.11	0.09	Meat Meal Tankage
27444	8.03	0.08	0.05	Meat & Bone Scraps
27447	11.47	0.13	0.09	Fish Meal
27449	8.79	0.07	0.05	Meat & Bone Scraps
27452	7.51	0.07	0.05	Meat, Bone, & Hoof Scraps
27492	9.06	0.13	0.12	Meat and Bone Scraps
27510	8.93	0.14	0.14	Meat Scraps
27536	10.16	0.16	0.15	Tankage
27548	9.14	0.08	0.06	Meat Scraps
27551	12.65	0.12	0.10	Blood Meal
27555	8.72	0.13	0.11	Meat & Bone Scraps
27559	7.96	0.08	0.05	Meat & Bone Scraps
27576	8.38	0.17	0.17	Meat & Bone Scraps
27577	9.01	0.08	0.06	Meat Scraps
27582	8.85	0.17	0.16	Meat & Bone Scraps
27696	8.23	0.07	0.06	Meat Scraps

* Average of two or more determinations.

TABLE 2.—*Determination of known amounts of urea added to feeds*

SAMPLE	GRAMS UREA ADDED TO 1 GM. SAMPLE	UREA FOUND (AFTER CORRECTION FOR AMOUNT IN TABLE 1)*
Meat and Bone Scraps (27002)	0.025	0.025
	0.010	0.010
	0.005	0.005
Digester Tankage (27065)	0.025	0.025
	0.010	0.010
	0.005	0.005
Meat and Bone Scraps (27154)	0.025	0.025
	0.010	0.010
	0.005	0.005
Meat Scraps (27510)	0.025	0.025
	0.010	0.010
	0.005	0.005
Steamed Bone Meal (27063)	0.025	0.025
	0.010	0.010
	0.005	0.005
Fish Meal (27447)	0.025	0.025
	0.010	0.010
	0.005	0.005
Blood Meal (27551)	0.025	0.025
	0.010	0.010
	0.005	0.005

* Average of two to eight determinations.

content of feeding stuffs and the amount of nitrogen as determined by the methods herein outlined for ammoniacal nitrogen and urea plus ammoniacal nitrogen.

As a test of the method, known amounts of urea were added to several of the samples included in Table 1 and the urea determined. These data (Table 2) are in most cases the average of multiple determinations. The replication of determinations was made to overcome analytical error caused mainly by the difficulty in obtaining a representative sample.

SUMMARY AND DISCUSSION

The amount of urea and ammoniacal nitrogen normally present in packing house products is variable. Analysis of 47 such samples gave an average nitrogen content of 0.10 per cent and a maximum of 0.19. Similar analyses for urea only, obtained by differences, gave an average nitrogen content of 0.02 per cent and a maximum of 0.06. The corresponding figures

for ammoniacal nitrogen were 0.07% and 0.18%. In determining the amount of urea added to packing house products, it is suggested that a tolerance of 0.10% nitrogen is sufficient allowance for such nitrogen as normally appears in such feeds.

The present official A.O.A.C. method for urea and ammoniacal nitrogen was found to give low urea results in a few cases. It is suggested that it be modified to include the use of the phosphate buffer. Thus revised, the method has been found to give accurate results over a wide range of conditions. It might be further studied with regard to the use of calcium chloride to control the distillation of carbon dioxide. Other factors, such as optimum size of sample, should be considered.

REFERENCES

(1) Official Methods of Analysis, A.O.A.C., 7th Ed., 1950, p. 345 (22.22); *This Journal*, 27, 89 (1944).
(2) "Practical Methods in Biochemistry." 3d Ed. Williams and Wilkins Co.

CHROMATOGRAPHIC PROPERTIES OF OIL-SOLUBLE COAL-TAR COLORS

By Louis C. Weiss (Food and Drug Administration, Federal Security Agency, Los Angeles, Calif.)

The "Coal Tar Color Regulations" (1) list 117 colors which may be certified for use in foods, drugs, or cosmetics. Certain of these may be used in all three classes of products. Others are restricted to use in drugs and cosmetics while still others may be used only in preparations intended for external application. The task of isolating and identifying individual colors, when occurring either singly or in mixtures, is one for which no complete systematic solution has as yet been developed. Koch (2) has devised an excellent procedure, which quite sharply divides the entire list into eight groups of from three to thirty colors each. The Methods of Analysis of the A.O.A.C. (3) provides methods for the identification of many of the dyes certifiable for use in foods, but little has been reported on most of the D&C and Ext. D&C colors.

Chromatography has been applied to the analysis of mixtures of a number of water soluble colors by Ruggli and Jensen (4, 5), and to both water- and oil-soluble colors by Ruiz (6, 7). The technique is ideally suited to this problem because no highly reactive reagents which might permanently alter the structure of the colors are used. Quantitative recoveries are generally possible, and only small, conveniently handled quantities of material are required.

However, methods for the isolation and identification of oil-soluble colors are few and not very satisfactory. This paper describes certain chromatographic properties and methods of separation of the 17 certi-

fiable colors which may be classed as oil-soluble and which constitute group 8 in the Koch system. Five non-permitted colors are also included.

The colors used were commercial samples and were not purified before use. Color solutions were prepared by dissolving weighed samples in appropriate solvents to give concentrations of 0.2 mg/ml. Ligroin was used as the solvent wherever possible. In some cases other solvents were used when the color was not sufficiently soluble in ligroin. Some of the

TABLE 1.—*Preliminary chromatographic separation of oil-soluble dyes*

COLOR	SOLVENT	CONCENTRATION (mg/ml)
FD&C Yellow No. 4	Ligroin	.20
FD&C Yellow No. 3	Ligroin	.20
D&C Red No. 30	Xylene	.12
D&C Violet No. 2	Ligroin	.20
Sudan I (C.I. 24)	Ligroin	.20
Butter Yellow (C.I. 19)	Ligroin	.20
FD&C Red No. 32	Ligroin	.20
FD&C Orange No. 2	Ligroin	.20
D&C Green No. 6	Ligroin	.20
D&C Red No. 36	CCl_4	.10
Aminoazotoluene (C.I. 17)	Ligroin	.20
D&C Red No. 18	Ligroin	.20
D&C Red No. 17	Ligroin	.20
Ext. D&C Blue No. 5	Ligroin	.20
Aminoazobenzene	Ligroin	.20
Ext. D&C Yellow No. 5	CCl_4	.08
D&C Orange No. 17	Xylene	.08
D&C Red No. 35	CCl_4	.08
Ext. D&C Orange No. 1	CCl_4	.20
D&C Red No. 38	Xylene	.076
Sudan G (C.I. 23)	Ligroin	.072
D&C Yellow No. 11	CCl_4	.20

colors could not be prepared in the above concentration in any of the usual solvents. Saturated solutions of these colors were prepared and the concentration determined by evaporating a portion to dryness and weighing the residue. The solvent used and the concentration of each color is shown in Table 1.

All columns in this work were packed to a height of about 15 cm. The volume of solvent required to wet the column completely is designated as V. This is a convenient device since the diameter of the column can be varied without altering the effect of a given number of "V's" of developing solvent. It must be determined experimentally for columns of various sizes. Thus, 0.5 mg of each color was chromatographed on each of four separate silicic acid columns and each chromatogram developed with

2 V of one of four benzene-ligroin mixtures. The results are shown in graphic form in Figure 1. Each rectangle represents a single chromatogram and shows in idealized form the location and size of the color zone. The developers used are indicated. Arrows show that the color zone developed completely out of the column. The list is arranged in the order of increasing adsorbability.

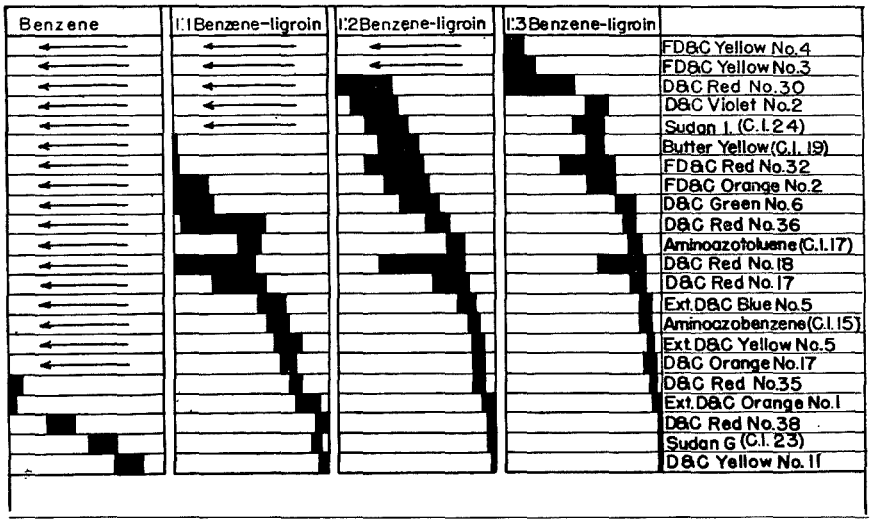

FIG. 1.

REAGENTS

(1) *Silicic acid adsorbent I.*—A mixture of 2 parts Merck's reagent grade silicic acid and 1 part Johns-Manville Hyflo-Supercel by weight. After thorough mixing the material must be passed through a 50 or 60 mesh screen and again mixed.

(2) *Ligroin.*—Skellysolve B., B.P. 60°–70°, redistilled before use.

(3) *Benzene*—Reagent grade, thiophene-free, redistilled before use.

APPARATUS

1. *No. 2 chromatographic tube,* similar to Scientific Glass Apparatus Company No. J-1661.

2. *Pusher, a wooden rod* somewhat longer than the tube, which will fit loosely in above.

PROCEDURE

The tube is packed by first tamping a wad of cotton on the perforated plate with the pusher and then slowly filling with adsorbent to a height of ca 17 cm, while applying suction with an aspirator or vacuum pump.

After vigorously tapping the tube over its entire length to further compact the adsorbent, the surface is smoothed with the pusher by gentle tamping and finished off by tapping the tube till the surface is smooth and level. The column should now be about 15 cm. in length.

A prewash of V of ligroin (see above) is added to the column. When nearly all the ligroin has been drawn into the column, the color solution is introduced. Small portions of developer are then added until all the color has been washed into the adsorbent. The remainder of the tube above the column can then be kept filled with developer. The surface of the column must always be covered with liquid and the suction must not be interrupted once the prewash has been added.

If the probable identity of a color is known and the problem is one of purification, the selection of the developer from Figure 1 should be such as to move the zone into the lower half or third of the column with 2 V or more of solution. This affords opportunity for more rapidly developing impurities to move below the color zone or entirely out of the column and separates the color from more strongly adsorbed substances which will remain on the column above it.

When development is complete and the last of the solvent has been drawn into the column, the suction should be continued until the adsorbent is observed to pull slightly away from the wall of the tube. The column can then be extruded with the pusher and the color zone cut out and eluted with absolute ether or absolute alcohol. If further purification is necessary the color can be rechromatographed.

When dealing with an unknown color, the proper developer must be determined by experiment. The behaviour of the unknown under standard conditions of development may give an important clue to its identity. Additional tests such as mixed chromatograms, spectral curves, and melting points serve as confirmatory tests.

In some instances the color develops in a wide diffuse band leaving a portion of the color at the top of the column. This is due to insolubility of the color in the developing mixture. That portion which does develop moves largely with the solvent in which it is added to the column. This is the case with D&C Red No. 30, D&C Orange No. 17, and D&C Red No. 36. The undeveloped zones are not shown.

A number of colors have been shown to be, or give evidence of being mixtures. The sample of D&C Violet No. 2 used in this work gave a blue and a yellow zone in addition to the principal violet band and appeared above the latter in that order. Only the violet band is shown in Figure 1. FD&C Red No. 32 is a mixture of isomers which are partly resolved by this procedure. The zone is shown on Figure 1 as an entity. The Ext. D&C Blue No. 5 contained a small amount of green color which remained at the top of the column.

It is possible to separate mixtures of many of the colors by the fore-

going procedure. If the composition of the mixture is known qualitatively, an inspection of Figure 1 will indicate whether or not the components can be separated and if so, what the developer should be. If, on the other hand, the identity of the components is not known, a preliminary chromatogram will eliminate many of the colors as possible components of the mixture.

However, it is obvious from Figure 1 that many possible mixtures can-

TABLE 2.—*Final chromatographic separation of pairs of oil-soluble dyes*

COLOR	ADSORBENT	DEVELOPER	DEGREE OF SEPARATION*
FD&C Yellow No. 4 / FD&C Yellow No. 3	Silicic acid III	3½ V 10% ether-ligroin	Fair
D&C Red No. 30 / D&C Violet No. 2	Alumina	1 V xylene	Excellent
Sudan I (C.I. 24) / Butter Yellow (C.I. 19)	Silicic acid III	.75 V xylene	Excellent
FD&C Red No. 32 / FD&C Orange No. 2	Magnesia	1 V benzene	Excellent
D&C Green No. 6 / D&C Red No. 36	Magnesia	.75 V benzene	Excellent
Aminoazotoluene (C.I. 17) / D&C Red No. 18	Magnesia	2 V 1+2 ether-ligroin	Good
D&C Red No. 17 / Ext. D&C Blue No. 5	Magnesium silicate	2 V 10% ether-pet. ether†	Fair
Aminoazobenzene (C.I. 15) / Ext. D&C Yellow No. 5	Magnesia	2 V 1+1 benzene-ligroin	Good
D&C Orange No. 17 / D&C Red No. 35	Silicic acid II	1 V 5% ether-ligroin	Excellent
Ext. D&C Orange No. 1 / D&C Red No. 38	Silicic acid II	2½ V 1+1 benzene-ligroin	Excellent
Sudan G (C.I. 23) / D&C Yellow No. 11	Magnesia	2 V 1+1 $C_2H_4Cl_2$-ligroin	Fair
	Silicic acid III	1½ V 1+1 $C_2H_4Cl_2$-ligroin	Fair
	Magnesia	1½ V 1+1 $C_2H_4Cl_2$-ligroin	Excellent
	Magnesia	1 V 1+3 $C_2H_4Cl_2$-ligroin	Excellent
	Magnesia	1 V 1+1 $C_2H_4Cl_2$-ligroin	Good
	Silicic acid II	1 V 1+1 $C_2H_4Cl_2$-CCl_4	Good
	Talc	3 V xylene	Fair
	Silicic acid II	2 V 1+1 $C_2H_4Cl_2$-ligroin	Fair
	Silicic acid I	1½ benzene	Excellent
	Silicic acid I	2 V benzene	Excellent
	Silicic acid II	3 V benzene	Excellent

* "Fair" indicates that interzone may not be clean and zones are not widely separated. Re-chromatographing is usually necessary for quantitative results.
"Good" indicates that the interzones are clean but not very wide.
"Excellent" indicates the presence of wide clean interzones.
† The prewash for this column should be petroleum ether rather than ligroin.

not be resolved by this procedure. If such a mixture is suspected, chromatograms which result in more effective resolution may be run. By the use of other adsorbents and developers, every adjacent pair of colors on Figure 1 has been separated. The color combinations, adsorbents, and developers are presented in Table 2.

Reagents

(1) *Silicic acid adsorbent II.*—A mixture of 4 parts Merck's reagent grade silicic acid with 1 part Johns-Manville Hyflo Supercel by weight.

(2) *Silicic acid adsorbent III.*—Mallinckrodt Acid Silicic, 100 Mesh (For Chromatography).

(3) *Activated alumina adsorbent.*—A mixture of 2 parts Alorco Activated Alumina, F-20 grade, ground to −200 mesh and 1 part Hyflo Supercel by weight.

(4) *Magnesium oxide adsorbent.*—A mixture of 1 part Micron Brand No. 2641 Magnesia and 1 part Hyflo Supercel by weight.

(5) *Talc adsorbent.*—A mixture of 2 parts Baker and Adamson Talcum Powder USP and 1 part Hyflo Supercel by weight.

(6) *Magnesium silicate adsorbent.*—Magnesol, dry cleaning grade 80-90%-100 mesh. Westvaco Chemical Division, Food Machinery and Chemical Corp.

(7) *Ethyl ether, reagent grade, absolute.*

(8) *Ethylene dichloride,* B.P. 83.5°–85°C., redistilled from technical grade.

(9) *Carbon tetrachloride,* reagent grade, redistilled before use.

(10) *Xylene,* reagent grade, redistilled before use.

(11) *Petroleum ether, B.P.* 30°–60°, redistilled before use.

(Only the magnesium oxide and alumina adsorbents need be stored in airtight containers; clean sections of silicic acid columns may be air dried and re-used. Several lot numbers of Merck's silicic acid have been used and found to be quite uniform in adsorptive characteristics. It is a light, almost impalpable powder, mostly −200 mesh. The only unsatisfactory lot ever encountered was of much coarser particle size. All adsorbent mixtures must be sieved as previously described, and the technique of packing the column and adding reagents does not vary.)

DISCUSSION

Many of the bands depicted in Figure 1 are somewhat diffuse with uneven boundaries which are impossible to locate precisely. Also, minor variations in adsorptive capacity and column density are apt to occur. For these reasons the positions of the zones as shown on the chart must be regarded as qualitative only; no calculation of R_f values should be attempted from these data.

Several of the separations described in Table 2 are imperfect as the

interzones are not clean. When quantitative results are required in these instances, the extruded column should be cut at the cleanest part of the interzone, the two parts eluted and separately re-chromatographed. These chromatograms can then be divided in the same way and the eluates of like fractions combined. In this way quantitative separations usually can be made.

A number of the red and orange dyes used in this study contain nitro groups. These colors are adsorbed very strongly by the alkaline adsorbents. When adsorbed they change to purple or blue in color, and require a strong devloper to move them down the column. However, they can be recovered unchanged upon elution. This is a valuable aid in identifying unknown colors. Alkaline adsorbents such as alumina and magnesia also have a very strong affinity for phenolic OH groups. Acidic adsorbents do not appear to have so pronounced an effect on NH_2 groups.

The isolation of the color or colors from the materials in which they are found is not always a simple matter and no generally applicable procedure has been found. If a petroleum ether extract can be prepared which does not contain gross quantities of fats or hydrocarbon oils, chromatographing on a silicic acid column is usually effective. If the residue after elution and evaporation indicates the presence of contaminants, re-chromatographing on another adsorbent should be tried.

Petroleum ether solutions of fats and oils should be chromatographed on an alumina column. By developing with $1\frac{1}{2}$ V of petroleum ether and then extruding and eluting, the bulk of the oil will be removed. Re-chromatographing will then give a fairly clean residue.

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SPECTROPHOTOMETRIC DETERMINATION OF BETA-CAROTENE STEREOISOMERS IN ALFALFA

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The recent development of a chromatographic procedure for the determination of isomeric fractions of β-carotene (2) and the spectrophotometric characterization of the three most abundant stereoisomers (4) provide the background for development of a spectrophotometric method of analysis for β-carotene stereoisomers. Such a method is presented here with a procedure for its application to alfalfa. The method should be applicable to suitably prepared extracts of β-carotene from any source, but for each source a procedure must be established for preparing the extracts free of impurities which may interfere with the spectrophotometric measurements. The spectrophotometric method eliminates the need for hydrated lime having closely specified adsorption properties (2) and is considerably faster than the chromatographic procedure.

Beadle and Zscheile (1) described a spectrophotometric analysis of β-carotene extracts into two components which subsequent work (5) has revealed consisted primarily of neo-β-carotene-B and a mixture of all-trans-β-carotene and neo-β-carotene-U. Since approximately 95 per cent of β-carotene occurs as the all-trans, neo-B, and neo-U isomers in an equilibrium mixture at 25° (2, 5), β-carotene solutions are treated here as containing three light-absorbing components with absorption curves essentially those of the pure isomers (4) shown in Figure 1. The similarity of these three curves indicates that an analytical method based on the differences in the absorbtivities of the three isomers will require precise spectrophotometric measurements.

Three successive points were considered in the development of the spectrophotometric procedure: (a) Can spectrophotometric measurements readily be made with sufficient precision to give reproducible analytical results for mixtures of the neo-B, all-trans, and neo-U isomers of β-carotene? (b) Can absorption constants be found such that spectrophotometric analyses of β-carotene solutions agree with results obtained by the chromatographic procedure (2) when the latter is used as a reference method? (c) What treatment is required in the preparation of β-carotene extracts of alfalfa to remove light-absorbing impurities which would interfere in the spectrophotometric method?

SPECTROPHOTOMETRIC CONSIDERATIONS

Spectrophotometric analysis of a solution containing three light-

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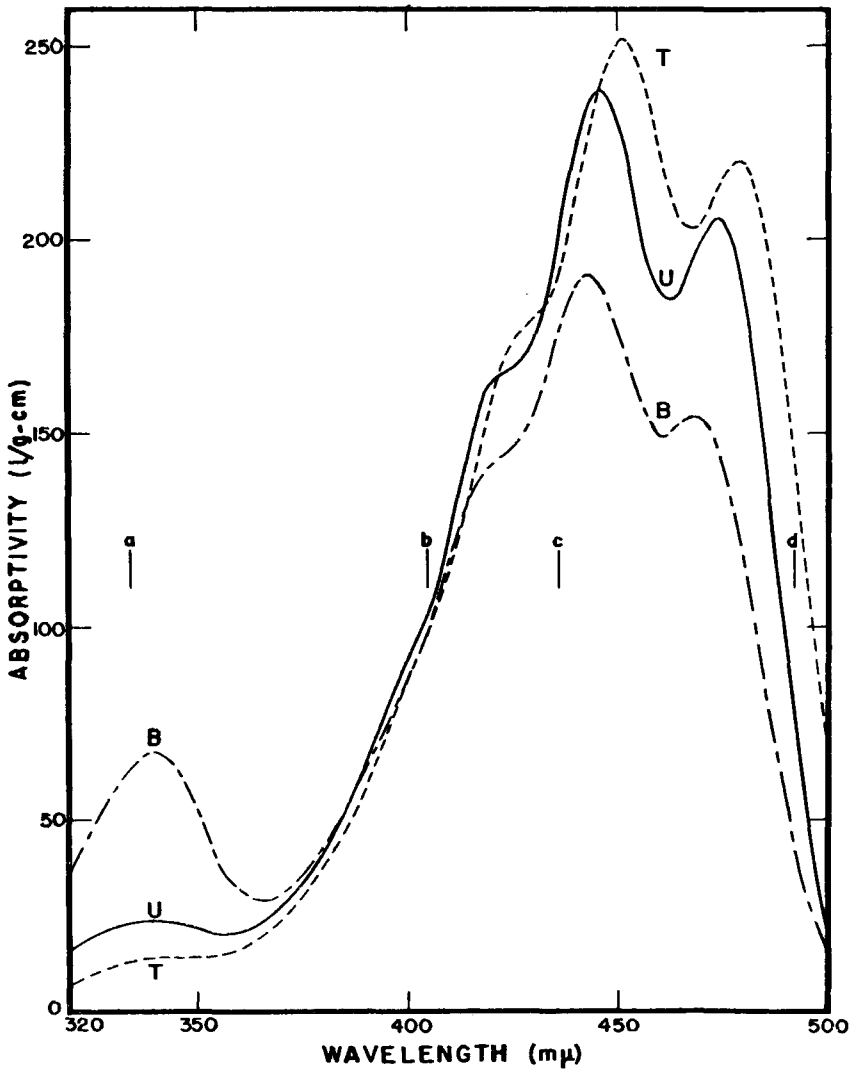

FIG. 1.—Absorption spectra of three stereoisomers of beta-carotene (4). B = neo-β-carotene-B; U = neo-β-carotene-U; T = all-trans-β-carotene. a,b,c,d indicate the location of mercury arc lines 334.1, 404.7, 435.8, and 491.6 mμ respectively.

absorbing components obeying Beer's Law requires absorbancy measurements at three different wavelengths and a knowledge of the absorptivities of the three pure components at each of the wavelengths. Mathematically, for measurements reduced to the same path length (1 cm.) and dilution, we may write three simultaneous equations expressing the fact that the absorbancy at each wavelength is the sum of the absorbancies of the individual components:

$$\begin{aligned} A_1 &= \alpha_{b1}B + \alpha_{t1}T + \alpha_{u1}U \\ A_2 &= \alpha_{b2}B + \alpha_{t2}T + \alpha_{u2}U \\ A_3 &= \alpha_{b3}B + \alpha_{t3}T + \alpha_{u3}U \end{aligned} \qquad (1)$$

where A = absorbancy (optical density) at wavelength indicated by subscript; B, T, U = concentrations of neo-B, all-trans, and neo-U isomers respectively; α = absorptivity for isomer and wavelength indicated by subscripts; subscripts 1, 2, 3 refer to wavelengths and b, t, u refer to isomers. The solution of Equations (1) for B, T, U gives:

$$\begin{aligned} B &= C_{b1}A_1 + C_{b2}A_2 + C_{b3}A_3 \\ T &= C_{t1}A_1 + C_{t2}A_2 + C_{t3}A_3 \\ U &= C_{u1}A_1 + C_{u2}A_2 + C_{u3}A_3 \end{aligned} \qquad (2)$$

where the C values are the appropriate algebraic combinations of the α values of Equations 1.

Maximum precision in spectrophotometric analysis is usually obtained by choosing wavelengths for absorbancy measurements such that at each wavelength (1) a different component absorbs strongly compared to the other components and (2) the absorption curve of the strongly absorbing component is not steep. It is seen from Figure 1 that a wavelength in the cis-peak region near 340 mμ meets both these conditions. Despite the steepness of the absorption curves near 490 mμ, a second wavelength is picked in this region because the all-trans isomer absorbs strongly relative to the neo-U and neo-B isomers. The choice of the third wavelength is not clearly indicated, but the criteria mentioned above suggest 470, 445, and 425 mμ as best possibilities.

Errors in spectrophotometric measurements are normally greatest at wavelengths at which the absorption curves of the components show steep slopes since a small error in wavelength setting or calibration produces a relatively large error in absorbancy. This source of error can sometimes be effectively eliminated by use of a line source of radiation for the sepctrophotometer (6). Fortunately the mercury arc has several strong lines in the region of interest, the positions of four of which are indicated in Figure 1. Measurements on the samples discussed below were made on a Beckman Model DU spectrophotometer at 490, 470, 445, 425, and 340 mμ with the tungsten filament source and at 334.1, 404.7, 435.8, and 491.6 mμ with the mercury lamp source normally used for checking the wavelength calibration of the instrument. Data were ob-

tained at these various wavelengths so that results calculated with different choices of wavelengths (Equation 1) could be compared. Measurements with the mercury arc source were made first on each sample; the wavelength dial readings thus obtained served to calibrate the wavelength scale of the instrument for succeeding measurements on the same sample with the tungsten filament source.

Results of detailed calculations are presented below for the following sets of wavelengths: I.—340, 470, 491.6 mμ; II.—334.1 435.8, 491.6 mμ; III.—340, 470, 490 mμ. Set I requires use of both sources, set II uses only the mercury arc source, and set III uses only the tungsten source. Since the mercury arc source is not as stable as the tungsten filament source, set I is expected to give the best results, since it uses the mercury arc source only where the absorption curve of the sample is steep.

Although 435.8 and 407.8 are not isolated single lines in the mercury spectrum, it was found experimentally that density readings of beta-carotene solutions on the Beckman DU spectrophotometer with the mercury arc source were not dependent on slit width for the lines 334.1, 435.8, and 407.8 mμ, but did vary appreciably with slit width for 491.6 mμ. In measurements reported here the slit width settings of Table 1 were used. Care must be used in making wavelength settings on mercury

TABLE 1.—*Slit widths used at various wavelengths*

SOURCE	TUNGSTEN FILAMENT					MERCURY ARC			
λ (mμ)	340	425	445	470	490	334.1*	404.7*	435.8*	491.6
Slit (mm)	0.10	0.025	0.020	0.020	0.015	0.200	0.03	0.02	1.00

* Slit width is unimportant for these lines. (See text.)

arc lines. After the slit has been set, the wavelength dial approximately set, and the sensitivity knob adjusted, the exact setting of the wavelength dial is made so as to give maximum intensity as indicated by the needle of the balance detection meter. This setting is facilitated by fastening an arm (a laboratory extension clamp may be used) to the wavelength dial so that the setting can be easily varied by small amounts.

Dilutions were made so as to give an absorbancy (optical density) between 0.6 and 0.8 at 470 mμ. A path length of 1 cm. was used, except in the cis-peak region where a path length of 5 cm. was used if the absorbancy for a 1 cm. path was less than 0.2. Iso-octane (2,2,4-trimethylpentane) was used as solvent. Since the presence of appreciable amounts of acetone contributes to absorption in the cis-peak region, it is essential that any acetone present be removed before spectrophotometric measurements are made. This is readily done by washing the isooctane solution five times with equal volumes of water in a separatory funnel.

Since the absorption coefficients obtained earlier (4) on the pure isomers with a tungsten filament source could not be considered accurate for use with a monochromatic source such as the mercury arc, chromatographically homogeneous solutions of neo-B, neo-U, and all-trans isomers in iso-octane were prepared and their relative absorptions were determined at the wavelengths mentioned above for each source and at the wavelength of maximum absorption. These relative values were converted to absolute ones for each isomer by use of the value obtained earlier on

TABLE 2.—*Absorptivities (l/g-cm) for iso-octane solutions of β-carotene isomers**

SOURCE	TUNGSTEN FILAMENT						MERCURY ARC			
λ (mμ)	λ(max)	340	425	445	470	490	334.1	404.7	435.8	491.6
neo-B	(191)	80	145	190	154	55	80	97	174	48
all-trans	(251)	13.8	173	238	206	155	13.1	98	193	135
neo-U	(238)	22.6	168	237	203	86	21.9	104	200	75

* Based on relative measurements at the various wavelengths and on the absolute values at λ_{max} found in Reference 4.

solutions of the crystalline isomer (4) at the wavelength of maximum absorption. The results are shown in Table 2. The figures are in satisfactory agreement with the curves published earlier (4) except for the absorption in the cis-peak region of the neo-B isomer. Five different neo-B solutions prepared in the present study all showed cis-peak absorption 20 to 25 per cent higher relative to the principal absorption peak than was found with solutions of the crystalline isomer (4).

ANALYSIS OF β-CAROTENE SOLUTIONS

Two separate groups of β-carotene solutions of known isomer composition were prepared and measured to provide data to answer the first two questions posed in the introduction with regard to the feasibility of obtaining spectrophotometric results of adequate precision and accuracy. These standard solutions were prepared by mixing measured volumes of stock solutions of the all-trans, neo-B, and neo-U isomer fractions.

The stock solutions were prepared in the following way: An iodine isomerized iso-octane solution of crystalline β-carotene was chromatographed on a 7.5 cm ×25 cm $Ca(OH)_2$ column using 1.5% para-cresyl methyl ether as developer. The developer was washed out with iso-octane, the column was extruded, and the three bands corresponding to neo-B, all-trans, and neo-U fractions (4) were removed. Each fraction was eluted with ethanol, transferred to iso-octane, placed on a small magnesia column, washed with iso-octane, and again eluted with ethanol. The ethanol was washed out with water and the water was removed with sodium sulfate.

Each of the stock solutions was analyzed chromatographically (2) for traces of the other two principal isomers and its concentration determined by its absorbancy at the wavelength of maximum absorption using the coefficients of the earlier work (4). The compositions of the mixtures, calculated from the volumes of mixing and the compositions of the isomer fractions, are labeled *Std.* in Table 3. These figures were confirmed by the chromatographic analyses (2) of the mixtures shown in Table 3 under the symbol *Chrom.* Eight mixtures were prepared in the first such experiment and six in the second as indicated in Table 3. Spectrophotometric measurements were made on all solutions as described in the previous section.

When the appropriate absorption coefficients of Table 2 were used in Equations 1 and 2 for calculating the composition of the mixtures for the

TABLE 3.—*Spectrophotometric analyses of β-carotene isomer mixtures*

SOLUTION NO.	METHOD	COMPOSITION (mg/l)			PERCENTAGE COMPOSITION		
		B	T	U	B	T	U
1-1	Std.	13.25	12.55	6.05	41.7	39.2	19.1
	Spec. I	13.10	12.35	6.05	41.6	39.2	19.2
	Chrom.	13.60	12.45	6.50	41.9	38.0	20.0
1-2	Std.	13.35	18.30	3.20	38.3	52.5	9.1
	Spec. I	13.50	18.10	2.55	39.6	52.9	7.5
	Chrom.	12.30	18.50	3.65	35.7	53.7	10.5
1-3	Std.	6.80	17.90	6.05	22.1	58.2	19.8
	Spec. I	7.05	17.65	5.50	23.3	58.5	18.2
	Chrom.	7.25	17.10	6.65	23.5	55.1	21.4
1-4	Std.	19.85	12.95	3.20	55.0	36.0	8.9
	Spec. I	20.20	13.30	2.40	56.2	37.1	6.7
	Chrom.	20.80	12.85	3.45	55.9	34.7	9.4
1-5	Std.	19.75	7.20	6.05	59.8	21.7	18.4
	Spec. I	19.20	7.25	6.50	58.3	21.9	19.8
	Chrom.	18.55	8.05	6.05	56.8	24.6	18.5
1-6	Std.	6.70	12.15	8.90	24.2	43.7	32.1
	Spec. I	6.20	11.85	9.20	22.8	43.5	33.7
	Chrom.	6.65	12.05	8.25	24.7	44.8	30.6
1-7	Std.	13.20	6.75	8.90	45.7	23.4	30.9
	Spec. I	13.15	6.75	9.05	45.4	23.3	31.3
	Chrom.	13.05	7.20	8.50	45.4	24.9	29.6
1-8	Std.	6.90	23.65	3.20	20.4	70.1	9.5
	Spec. I	6.65	23.15	2.65	20.5	71.4	8.1
	Chrom.	7.10	21.60	2.80	22.5	68.7	8.9
2-1	Std.	16.90	19.80	4.45	41.1	48.1	10.8
	Spec. I	16.50	19.50	5.30	40.0	47.2	12.8
	Chrom.	17.95	19.05	4.30	43.6	46.0	10.4
2-2	Std.	8.80	23.10	8.50	21.8	57.1	21.1
	Spec. I	8.70	22.25	9.45	21.5	55.1	23.4
	Chrom.	10.40	22.75	8.10	25.2	55.2	19.7
2-3	Std.	12.70	15.80	12.55	31.0	38.5	30.5
	Spec. I	12.50	16.20	12.25	30.6	39.6	29.8
	Chrom.	13.85	15.75	11.70	33.6	38.0	28.3
2-4	Std.	24.80	8.95	8.45	58.8	21.2	20.0
	Spec. I	24.75	10.20	7.90	57.8	23.8	18.4
	Chrom.	25.60	9.15	8.65	59.0	21.0	20.0
2-5	Std.	8.90	26.85	4.50	22.1	66.7	11.2
	Spec. I	9.05	26.30	4.80	22.6	65.5	11.9
	Chrom.	9.40	27.30	4.85	22.6	65.7	11.6
2-6	Std.	24.90	12.75	4.45	59.2	30.2	10.6
	Spec. I	24.55	12.50	5.05	58.3	29.7	12.0
	Chrom.	25.35	12.25	4.70	60.0	29.0	11.1

wavelength sets mentioned above, poor results were obtained, despite the fact that the observed absorbancies of the mixtures agreed well with the values calculated from the absorbancies of the stock isomer solutions and the volumes of mixing. This suggested that the coefficients of Table 2 obtained from measurements on solutions of relatively pure single isomers were not suitable for the isomer fractions as determined in the chromatographic procedure (2). The absorption coefficients for wavelengths of set I were then altered to produce approximately the best agreement possible between spectrophotometrically calculated compositions and the known compositions of the isomer mixtures as shown in Table 3 under the label *Spec. I*. The values of the adjusted coefficients in Equations 1 and 2 for wavelength set I are shown in Tables 4 and 5, respectively.

TABLE 4.—*Empirically adjusted absorptivities (l/g-cm) for iso-octane solutions of β-carotene isomers*

SOURCE	TUNGSTEN FILAMENT			MERCURY ARC			
λ (mμ)	340	470	490	334.1	404.7	435.8	491.6
neo-B	69.7	146.9	40.0	69.9	106.1	182.6	37.2
all-trans	14.80	205.3	150.2	14.16	98.5	192.0	132.3
neo-U	24.55	199.9	76.9	23.73	111.1	206.5	67.1

TABLE 5.—*Coefficients for equation 2*

λ SET	I			II			III		
λ(mμ)	340	470	491.6	334.1	435.8	491.6	340	470	490
B	20.131	−3.580	3.305	21.542	−3.266	2.434	20.307	−3.636	2.969
T	3.834	−5.971	16.395	6.806	−5.674	15.065	4.708	−6.244	14.729
U	−18.731	13.766	−19.267	−25.377	13.007	−16.160	−19.757	14.087	−17.308

Isomer concentrations will be in mg/l.

A similar empirical adjustment of absorption coefficients was made for wavelength sets II and III to produce agreement between spectrophotometrically calculated and known compositions of the isomer mixtures, in each case retaining the values of the adjusted coefficients already found for wavelength set I at those wavelengths common to set I and either set II or III. The additional adjusted coefficients thus found are included in Table 4 and the appropriate coefficients for Equations 2 are included in Table 5.

Table 6 presents a concise summary of the analyses of the mixtures of Table 3 by the different procedures. The symbols *Std. Chrom*, and *Spec. I* refer here to the isomer percentage composition values shown in Table 3. *Spec. II* and *Spec. III* refer to similar values calculated spectro-

photometrically using the data obtained at wavelength sets II and III, respectively, and the appropriate coefficients of Tables 4 and 5. The entries in Table 6 refer to the difference in percentage of the indicated isomer found by the procedures listed in the first column of the table for the fourteen mixtures of Table 3. Thus an entry of zero in the left-hand portion of the table indicates that on the average the isomer percentage found by the two procedures was the same, whereas the entries in the right-hand portion are mean values of the difference without regard to sign.

The data of Tables 3 and 6 show that spectrophotometric results can be made to agree closely with chromatographic analyses. Wavelength

TABLE 6.—*Comparison of isomer analyses of β-carotene solutions by various procedures*

PROCEDURES	DIFFERENCES IN ISOMER PERCENTAGE					
	AVERAGE DIFFERENCE			MEAN ABSOLUTE DIFFERENCE		
	B	T	U	B	T	U
(Chrom.)—(Std.)	0.70	−0.34	−0.03	1.5	1.5	0.9
(Spec. I)—(Std.)	−0.19	0.15	0.07	0.8	0.8	1.4
(Spec. I)—(Chrom.)	−0.85	0.66	0.01	1.7	1.6	1.9
(Spec. II)—(Std.)	−0.08	0.32	−0.25	0.9	1.2	1.5
(Spec. III)—(Std.)	−0.38	−0.57	0.96	0.7	1.9	2.1

sets I, II, and III are all feasible for spectrophotometric analyses and rank in that order on the basis of reliabiltiy and precision. We also noted that the wavelength dial readings for the mercury arc lines showed little variation in a three-month period on our spectrophotometer. If an average corrected dial setting had been used for each of the wavelengths in set III, the entries for the last row of Table 6 would have been slightly improved.

ANALYSIS OF ALFALFA EXTRACTS

Extracts of alfalfa meal were prepared by soaking 25 g. meal in 100 ml. iso-octane for 15 minutes in the dark (3). These extracts were then passed through a 2 cm.×10 cm. magnesia column to remove chlorophylls and xanthophylls, the carotene was eluted with 10% acetone in iso-octane and the acetone was removed by five successive washes with equal volumes of water. Spectrophotometric and chromatographic analyses of the resulting β-carotene solution agreed for some meals and not for others, presumably due to the presence in some cases of appreciable quantities of impurities which absorbed radiation at one or more of the wavelengths used in the spectrophotometric measurements. Extracts of three selected meals of different carotene content were then adsorbed

TABLE 7.—*Isomer analyses of β-carotene extracts of alfalfa meals*

SAMPLE*	ORIGIN	TOTAL CAROTENE P.P.M., DRY BASIS	PROCEDURE	ISOMER PERCENTAGE COMPOSITION		
				B	T	U
A	Brawley, Southern California	192	Chrom.	39.9	41.4	18.7
			Spec. I	37.7	39.9	22.4
			Spec. II	36.7	38.4	24.8
			Spec. III	38.6	45.4	15.7
B	McAllen, Southern Texas	143	Chrom.	41.0	46.1	12.9
			Spec. I	40.3	43.2	16.5
			Spec. II	40.3	43.1	16.6
			Spec. III	41.3	48.0	10.7
C	Gerber, Northern California	229	Chrom.	34.0	51.0	15.0
			Spec. I	35.6	52.1	12.3
			Spec. II	35.6	52.4	12.0
			Spec. III	36.4	56.1	7.5
D	Firebaugh, Central California	241	Chrom.	34.2	53.9	12.0
			Spec. I	37.6	55.6	6.8
			Spec. II	38.3	57.2	4.5
			Spec. III	38.1	58.2	3.7
E	Dixon, Central California	199	Chrom.	27.8	61.8	10.4
			Spec. I	29.8	61.9	8.3
			Spec. II	30.7	64.2	5.1
			Spec. III	30.9	67.4	1.7
F	Cozad, Nebraska	158	Chrom.	28.0	59.8	12.1
			Spec. I	31.6	59.1	9.3
			Spec. II	32.0	60.2	7.8
			Spec. III	31.7	59.8	8.5
G	Ryer Island, Central California	185	Chrom.	35.1	50.0	14.8
			Spec. I	36.1	52.6	11.3
			Spec. II	36.9	53.7	9.4
			Spec. III	36.0	51.8	12.2
H	Brawley, Southern California	260	Chrom.	37.5	44.1	18.4
			Spec. I	38.3	46.1	15.6
			Spec. II	38.1	46.2	15.7
			Spec. III	38.5	47.2	14.3
J*	Maxwell, Northern California	423	Chrom.	39.8	46.6	13.6
			Spec. I	39.2	46.9	13.9
			Spec. II	39.4	47.2	13.4
			Spec. III	39.4	47.8	12.8
K	LeRoy, New York	207	Chrom.	42.5	41.7	15.8
			Spec. I	41.4	45.7	12.9
			Spec. II	41.4	46.2	12.4
			Spec. III	41.4	45.5	13.1
L	Kansas City, Missouri	138	Chrom.	42.8	39.0	18.2
			Spec. I	41.4	39.7	18.9
			Spec. II	40.0	38.0	22.0
			Spec. III	41.6	41.1	17.3
M	Firebaugh, Central California	162	Chrom.	33.3	49.2	17.5
			Spec. I	43.6	42.0	14.4
			Spec. II	43.0	39.1	17.9
			Spec. III	44.1	44.7	11.2

* All samples were commercially dehydrated whole meals except J, which was a leaf meal.

on magnesia and washed with various volumes of iso-octane prior to elution with acetone and washing as described above. It was found that the absorption spectrum of the extract purified in this way varied appreciably with the volume of iso-octane used to wash the carotene on the magnesia column, but that 500 ml. iso-octane sufficed to produce a purified extract which showed no further change if larger volumes of iso-octane were used. Accordingly the above procedure, including washing the adsorbed carotene with 500 ml. iso-octane, was adopted for preparation of extracts of alfalfa meals for spectrophotometric analysis.

Analyses by both the chromatographic and spectrophotometric methods were made on twelve different alfalfa meals originating from various sections of the country. The results of these analyses are presented in Table 7 and a summary of the differences by the two methods is shown

TABLE 8.—*Differences in chromatographic and spectrophotometric analyses of alfalfa extracts*

PROCEDURES	DIFFERENCES IN ISOMER PERCENTAGE					
	AVERAGE DIFFERENCE			MEAN ABSOLUTE DIFFERENCE		
	B	T	U	B	T	U
Spec. I—Chrom.	0.58	0.67	−1.25	1.7	1.6	2.7
Spec. II—Chrom.	0.62	0.21	−1.65	2.1	2.3	4.1
Spec. III—Chrom.	1.05	2.99	−4.04	1.7	3.0	4.0

in Table 8. In preparing the latter table sample M was not included since it was well over a year old. It is seen from Table 8 that *Spec I.* procedure gave somewhat smaller mean absolute differences than *Spec. II* or *Spec. III* and that *Spec. III* procedure showed the greatest tendency to give results systematically in error. The mean absolute differences in percentage isomer compositions between the chromatographic and *Spec. I* procedures is seen to be very similar for the alfalfa extracts and for the β-carotene solutions of Tables 3 and 6.

DISCUSSION AND RECOMMENDATIONS

Failure of the absorption coefficients determined for solutions of pure neo-B, all-trans, and neo-U isomers to give spectrophotometric results in good agreement with the isomer composition determined chromatographically is not surprising since the fractions as collected in the chromatographic procedure are known to contain small amounts of the less abundant isomers. However the coefficients of Table 4 are difficult to rationalize in terms of the small quantities of rarer isomers present and should be regarded simply as empirically adjusted values.

Due to the smaller chance of wavelength errors, the use of wavelength sets I or II for spectrophotometric measurements would seem more reli-

able than use only of the tungsten filament source. However, details for spectrophotometric analyses using wavelength set III have been presented since the greater convenience of using only the tungsten source may in some cases outweigh the larger errors likely to result. Even when only the tungsten source is used for routine analyses, wavelength dial settings should be used which correspond to correct wavelengths of 340, 470, and 490 mμ.

Since the spectrophotometric measurements and calculations require less than thirty minutes per sample, the spectrophotometric procedure is much faster than the chromatographic separation of the isomers on a lime column. However the chromatographic procedure must be considered more reliable since it is less likely to give erroneous results due to the presence of impurities absorbing radiation in the cis-peak region.

The recommended spectrophotometric procedure for stereoiosomer analyses of β-carotene in alfalfa meal is summarized in the following steps: (1) prepare extracts for spectrophotometric measurements as described in the preceding section, including the wash of adsorbed carotene with 500 ml. iso-octane; (2) dilute an aliquot with iso-octane to a concentration suitable for spectrophotometric measurements and read the absorbancies on a Beckman Model DU spectrophotometer at the three wavelengths chosen using the appropriate slit widths of Table 1; (3) calculate the isomer concentrations of the diluted aliquot in mg. per liter from the absorbancies for a 1 cm. cell length with Equations 2 and the appropriate coefficients of Table 5.

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SUMMARY

β-carotene in plant extracts consists almost entirely of a mixture of neo-B, all-trans, and neo-U stereoisomers which may be separated and determined by a chromatographic procedure. Because this procedure is relatively slow, a rapid spectrophotometric method, suitable for routine work, was sought. It was found that spectrophotometric results obtained with empirically adjusted absorptivity coefficients in a three-component assay agreed well with those obtained by the chromatographic method. Alternate sets of three wavelength points are suggested for the assay. Certain lines of the mercury arc were found useful for minimizing wavelength errors in the spectrophotometric measurements. A procedure is given for application of the method to alfalfa meals. The method should be applicable to extracts of β-carotene from any source, but for each source a procedure must be established for preparing the extracts free of impurities which may interfere with the spectrophotometric measurements.

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EXCHANGEABLE HYDROGEN AS DETERMINED BY VARIOUS PROCEDURES IN RELATION TO THE SOIL'S CAPACITY FOR CALCITE DECOMPOSITION

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Exchangeable hydrogen is of importance because it is the principal cause of soil acidity, and its determination affords an approximate measure of that illusory value known as the "lime requirement" of soils. The several concepts of lime requirement in relationship to exchangeable hydrogen content have been discussed in two recent papers (13, 16). This paper deals chiefly with the comparative merits of the procedures that are being used or have been proposed for the determination of exchangeable hydrogen content of soils. The procedures which will be considered here readily fall into three distinct categories:

1. Those based upon the replacement of the adsorbed hydrogen through repeated treatments with salt solution of monovalent or divalent cations, of *p*H 7. The replacing agents belonging to this class are: (a) Ammonium acetate, (b) Barium acetate, and (c) Paranitrophenol buffer.

2. Those based upon hydrogen replacement by means of electrolytes of *p*H higher than 7, usually of *p*H 8 to 8.3. In this group belong the following: (a) Triethanolamine-$BaCl_2$ buffer, *p*H 8.1, (b) $Ca(OH)_2$-air equilibration, *p*H 8.3, and (c) Calcite decomposition through natural contact with soils.

3. Those that occupy a position intermediate between the above groups, 1 and 2, and prescribe serial titrations with $Ca(OH)_2$ or $Ba(OH)_2$ to *p*H 7 of soil suspensions in water or salt solution. This group embraces: (a) $Ca(OH)_2$ in 0.4 *M* $CaCl_2$ (3), (b) $Ca(OH)_2$ in H_2O (2), and (c) $Ba(OH)_2$ in 0.5 *M* Ba-acetate (16).

The procedures for hydrogen replacement by means of electrolytes at *p*H values above 7 were devised because of the known inefficacy of neutral salts, such as $BaCl_2$ and $CaCl_2$ in the performance of this task. Certain workers propose that the hydrogen replacement should be effected at the *p*H level that prevails when the soil is in equilibrium with an excess

of limestone under natural conditions (1, 8, 10). In the present study typical methods of the several groups are compared with respect to precision and adaptability as routine procedures for determinations in large numbers.

BRIEF DESCRIPTIONS OF THE PROCEDURES STUDIED

A brief description of each method used is given here, because it is believed that in many instances the lack of precise description of procedures is responsible for discordance in the results obtained by different analysts.

Ammonium acetate procedure.—Ammonium acetate has been accepted as the most suitable agent for the extraction of exchangeable metal cations (calcium, magnesium, and potassium) and for the determination of the exchange capacity of soils. The content of exchangeable hydrogen is then determined conveniently as being the difference between exchange capacity and the sum of the metal cations. The vulnerable points of this procedure lie in the alcohol-washing to effect the removal of adherent ammonium acetate and in the distillation of the adsorbed NH_4 ion as ammonia. Nevertheless, high degree of reproducibility of results is obtainable through use of this method when the prescribed technique is adhered to strictly.

In this laboratory the procedure is as follows: Soil charge calculated to give 1 to 2 m.e. of exchange capacity—usually 20 g of air-dry soil of the silt loam type—is placed in a 250 ml Erlenmeyer flask and shaken with approximately 100 ml of normal ammonium acetate, at *p*H 7. The suspension is allowed to stand overnight and then filtered on 5.5 cm Büchner filter under slight suction, and residue is leached with small quantities of the ammonium acetate until the combined volume is about 500 ml. The filtrate is transferred to a 600 ml beaker and evaporated to dryness on a hot plate and the soil residue is washed with small (10 ml) quantities of 95 per cent ethyl alcohol to a volume of ca 150 ml. The well-drained soil residue is then transferred into a 500 ml Kjeldahl flask with the aid of a short-stemmed funnel, the bulk of the residue being forced from the Büchner funnel by inverting and blowing through the stem. The adhering material is wiped clean with pieces of wetted filter paper and the whole residue washed down with a little H_2O and the aid of a "policeman." The adsorbed NH_4 is distilled over in a 200-ml distillate into an excess of 0.1 *N* HCl; the distillate is back-titrated with 0.1 *N* NaOH, using methyl red as indicator. The net ml of acid used, divided by the weight of soil taken, and multiplied by 10, represents the base exchange capacity, as m.e. per 100 g of air-dry soil. A necessary feature of the NH_4 distillation technique is the complete displacement of air from the system before the introduction of the NaOH (14).

The evaporation residue from the acetate leaching is ignited in the electric furnace at 550°C.; cooled, dissolved by warming in an excess of

0.1 *N* HCl and back-titrated with 0.1 *N* NaOH, using methyl red as indicator. The net ml of acid used, divided by the weight of soil taken and multiplied by 10, equals the sum of metal cations, as m.e. per 100 g of air-dry soil. Although it is not difficult of execution, this technique is not adapted to the determination of exchangeable hydrogen on a large scale.

Paranitrophenol buffer procedure.—This method, proposed recently by Woodruff (17), is most attractive for its simplicity and adaptability for

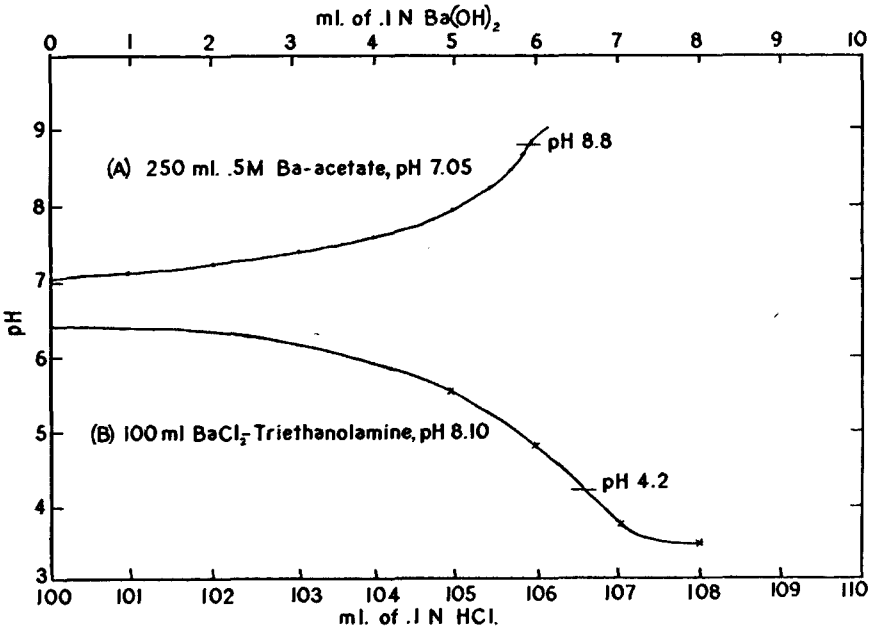

FIG. 1.—The buffer curves of (A) .5M Ca-acetate and (B) Triethanolamine-$BaCl_2$, which show the sensitive *p*H ranges and best end points for the respective titrations of replaceable hydrogen in soils. Upon basis of total extract from a 10-g. soil charge, each milliliter of acid or of base corresponds to 1 m.e. replaceable hydrogen per 100 g. of soil.

mass output. It consists of mixing an appropriate charge of soil with 10 ml H_2O and 20 ml of the buffer solution, and the determination of the *p*H value of the suspension after 30 minutes contact. The depression in the *p*H value from 7.0, divided by the soil charge and multiplied by 100, equals the exchangeable hydrogen, as m.e. per 100 g of air-dry soil. This test is applicable only to soils of *p*H 6.5 or below, and the soil charges are taken so that the *p*H in buffer suspension shall not fall below 6. The indicated value for exchangeable hydrogen is purported to determine the amount of limestone needed in order to raise the *p*H of a soil to 7.

Calcium acetate procedure.—In the use of Ba-acetate as the replacing agent for exchangeable hydrogen in soils the extraction technique is the same as that described for the use of ammonium acetate. Usually, the exchangeable hydrogen is determined by titration of the acetate leachate to the *p*H value of the extracting solution (11). A proposed modification of the procedure consists of the extraction of the exchangeable hydrogen with 0.5 *M* Ca-acetate, adjusted to *p*H 7, and the potentiometric titration of the extract to *p*H 8.8, with 0.1 *N* $Ba(OH)_2$. The buffer properties of the 0.5 *M* Ca-acetate against $Ba(OH)_2$ are given in the titration curve A of Figure 1.

Soil charges of 10 (or 20) g are shaken with approximately 100 ml of the Ca-acetate solution in 250 ml Erlenmeyer flasks and allowed to stand overnight. The suspensions are filtered through 12.5 cm folded filters that rest on 3-inch funnels, and the filtrates are caught in 250 ml volumetric flasks. The soil then is transferred onto the filter and is leached with small quantities of the Ca-acetate solution to a volume slightly below the 250 ml mark. The funnel is removed and Ca-acetate solution is added to the filtrate to bring it to mark. The solution is transferred and rinsed with H_2O into a 400-ml beaker and titrated with 0.1 *N* Ba $(OH)_2$ to *p*H 8.8 by using the larger type of glass electrode as indicator. This titration, in ml 0.1 *N* $Ba(OH)_2$, minus the titration value of 250 ml of the Ca-acetate blank, divided by the soil charge, and multiplied by 10, gives the exchangeable hydrogen in m.e. per 100 g of air-dry soil. Indicators may be used, but are not well adapted for accurate and rapid titration because of interferences caused by colored soil extracts.

The $Ca(OH)_2$-Air equilibration procedure.—Several variations have been introduced into the procedure that was proposed by Bradfield and Allison in 1933 (1). They stipulated the neutralization of the excess of $Ca(OH)_2$ by the passage of CO_2 prior to the final equilibration with air, and determination of residual carbonate. Naftel (10) modified the Bradfield and Allison procedure through use of the serial $Ca(OH)_2$ titration and designated the Ca supplied at the point of first maximal *p*H value after 18-hour air passage as the "Ca-sorption value," or the exchangeable hydrogen value of the soil. Mehlich (8) compared the residual carbonate of the Bradfield and Allison technique and a modification that comprised a serial titration. The Ca added, less that in solution at the initial constant *p*H value after 48-hour air passage was taken as the saturation point, or as a measure of the content of exchangeable hydrogen. The two procedures gave concordant results on 11 soils and soil materials.

In the present study the original "$CaCO_3$ equilibration" was employed as to residual carbonate determination, but the technique was different in other respects. Soil charges to give a maximum of 1.5 m.e. of exchangeable hydrogen were placed in 125-ml extraction flasks and a constant of 50 ml of .04 *N* $Ca(OH)_2$ was added to each flask. The flasks were con-

nected in series through inlet and outlet tubing and outdoor air was drawn through the suspensions for 48 hours. After determination of their *p*H values the suspensions were filtered on gravity filters and the alkalinity of the filtrates determined by titration with 0.1 *N* HCl. The filters and the lower end of the inlet tube then were returned to the original flasks, and the residual $CaCO_3$ determined by the steam distillation procedure

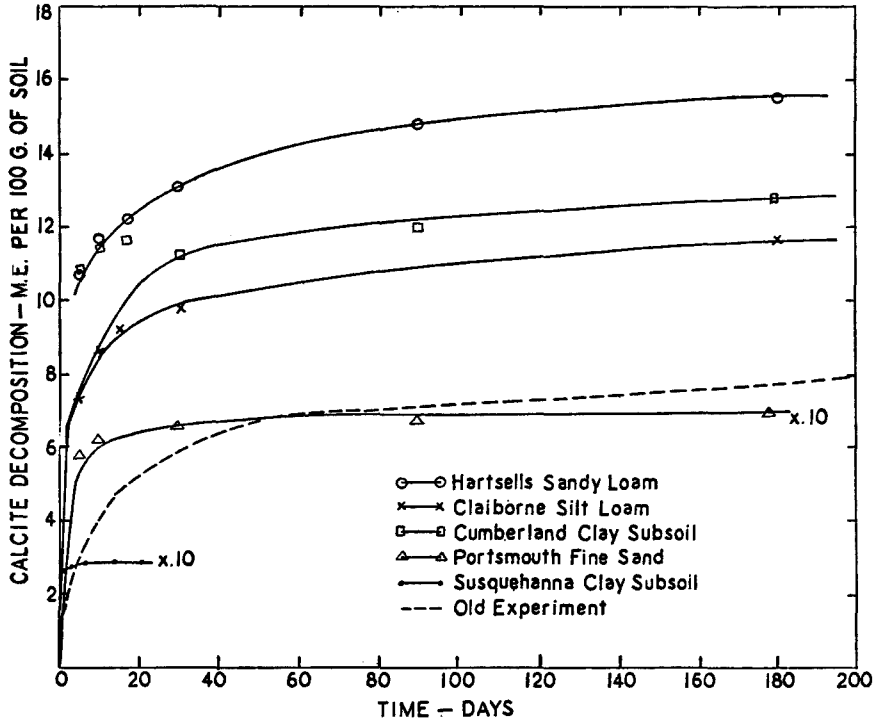

FIG. 2.—Progression in the decomposition of incorporated calcite, as effected by representative soils and subsoils, at 30°C. and 80 per cent moisture saturation.

(15). The $Ca(OH)_2$ addition, less the residual $CaCO_3$, and the titratable alkalinity of the filtrate, expressed as 0.1 *N* HCl, divided by the soil charge and multiplied by 10, represents the exchangeable hydrogen in m.e. per 100 g of soil.

The $BaCl_2$-triethanolamine buffer procedure.—Various versions of this procedure appeared from time to time since its original publication by Mehlich (7). In the present study the 1948 method has been adhered to with respect to the preparation of the replacing solution and the method of extraction and washing of the soil with $BaCl_2$ solution and with H_2O.

Because our objective was exchangeable hydrogen only, the entire extract was used in the determination of the hydrogen replaced in the conventional charge of 10 g of soil, whereas the original procedure calls for the titration of $\frac{1}{5}$ aliquot with .04 *N* HCl to the pink tint in the presence of an indicator mixture of bromcresol green and methyl red. The present authors believe that better definition of end-point is obtained through titration to *p*H 4.2, using the glass electrode. They also used 0.5 *N* HCl, in preference to the .04 *N* HCl, thus saving time and the titrant. The buffer capacity of the Triethanolamine $BaCl_2$ near its titration end-point against 0.1 *N* HCl is given in curve B, Figure 1.

The titer of the 100 ml of the buffer in ml of 0.1 *N* acid, less that of the unknown, divided by soil charge and multiplied by 10, registers the exchangeable hydrogen by this method in m.e. per 100 g of soil.

Calcite decomposition through natural contact with the soil.—This procedure for determination of exchangeable hydrogen, or the base-saturation capacity, of soils could come under the broad heading of "calcium carbonate equilibration methods." However, in studies under the general heading of "equilibration," during the past 20 years the soils were suspended in a solution of $Ca(OH)_2$ followed by equilibration of the system through passage of a current of outside air for periods of from 18 to 48 hours. The present procedure differs markedly from the techniques associated with the term "equilibration method" and, therefore, the designation of "calcite decomposition" or "soil-calcite reaction" is used to emphasize this distinction.

In earlier Tennessee Station experiments, decomposition of precipitated carbonates of $CaCO_3$ and $MgCO_3$, and other carbonates, were induced through their direct contact with soils. These investigations led to the observation " . . . that after more than satisfying the lime requirement according to the Veitch procedure, unleached and sterile soils were still able to effect, by ordinary contact, continued and appreciable decomposition of $CaCO_3$ with fixation of CaO, principally in the form of silicates" (6, p. 147). Moreover, these observations were based upon data that showed " . . . continued evolution of CO_2 from excessive treatments of $CaCO_3$ upon soils (where questions of biological influences and organic matter were eliminated) for a period of over two years . . . " (5, p. 573). The cited facts present a situation which is challenging in the face of any attempts of establishing a standard "base-saturated soil," whose definition is based upon the principle of equilibrium, or finite values of Ca-sorption (1, p. 64). Because of its fundamental nature and the potential practical value of its findings, the decomposition of calcite in soils has been investigated extensively in this laboratory during the past two years through the application of techniques that are featured by control of temperature and moisture variables, and by more precise methods of analysis.

The objectives of the presently reported investigation were (a) to establish conditions which favor maximal rapidity of reaction between calcite and acidic soils, (b) to determine whether the soil-calcite reaction could be advanced to equilibrium status within reasonable time limits under laboratory conditions, and (c) to establish the relationship between the soil-calcite decomposition values and the exchangeable hydrogen obtained through the use of a number of laboratory procedures.

Standard procedure for investigation of the soil-calcite reaction.—Charges of 100 g of 1-mm air-dry soils are placed in 150 ml beakers. (For soils of high content of organic matter and montmorillonitic clays, beakers of 250 ml size are more suitable.) Iceland spar, ground to pass a 325-mesh sieve, is weighed accurately in quantities of 10 to as high as 80 m.e. (0.5 g to 4 g) per experiment. The exact quantity is governed by the probable content of exchangeable hydrogen per 100 g of soil, and by amount necessary to maintain a residual $CaCO_3$ content of 5 to 15 m.e. One gram of calcite per 100 g of soil has been found most satisfactory for moderately acidic silt loams. With soil materials of unknown hydrogen content, it may be advisable to set up simultaneously, a number of mixtures of soil and a quantity range of calcite in order to obtain an indication as to analytically desirable rate of treatment. The calcite is triturated into a small portion (5–10 g) of the soil and the resultant mixture then is incorporated into the body of the charge by rolling it on a sheet of glazed paper. The soil-calcite mixture is returned to its container which then is tapped lightly. Distilled H_2O is added gradually to the extent of 80 per cent of the saturation capacity of the soil. The overall weight of the beaker is recorded and it is placed in a water bath at the desired temperature for the required length of time. Every 2 days the beaker is removed from the bath and restored to its original weight through addition of H_2O. At the end of the contact period the moist soil is removed from the beaker and spread out on a clean sheet of paper where it is allowed to air-dry, usually for a period of 24 to 48 hours. The soil is then ground to pass a 0.5 mm. sieve, thoroughly mixed, and placed in sealed container. Residual $CaCO_3$ is determined upon 10 g samples by means of the steam distillation method (15, p. 382) with approximately 15 ml of 0.1 *N* NaOH in the CO_2 absorption tube. The $BaCO_3$ suspension is allowed to stand 2 hours and the excess NaOH is titrated with 0.1 *N* HCl to a pale rose tint of the phenolphthalein indicator. A blank is obtained on the original soil. Occasionally soils of *p*H 6 to 6.5 were found to contain appreciable quantities of $CaCO_3$. On a 10 g charge the residual carbonate value, as m.e. per 100 g of soil, is obtained directly from the titration difference in ml of 0.1 *N* HCl, *i.e.*, blank soil titration, minus titration of unknown. The carbonate decomposition is registered through the difference between the calcite applied and the residual carbonate.

Simplified technique for soil-$CaCO_3$ reaction.—To overcome certain in-

adequacies in the standard procedure, a parallel technique was adopted. In this technique a 10 g charge of soil is properly mixed with the proportionate quantity of 325-mesh calcite and placed in 35 mm paraffine-coated soufflé cup. The mixture is wetted very gradually until the surface appears saturated. The cups are placed on an aluminum tray in a constant temperature water bath. The loss of water is restored daily, as judged by the eye. At the end of the contact period, the upper $\frac{1}{3}$ of the cup is cut off. The hard crust, if any, is loosened by a few jabs with the point of the scissors and the cup and its contents is then folded and slipped in the carbonate-decomposition flask. The remainder of the procedure is the same as described under the standard technique.

Here the chief advantage of the simplified technique is that it affords short-time calcite decomposition—24-hour or shorter periods—without the uncertainties as to the effect of the additional 24- to 48-hour drying period. The shorter technique also obviates the need for grinding and mixing of the sample, and thus avoids comparatively long delays in the analyses of short-time experiments. It has, however, a disadvantage in that the experimental mixture is used up entirely in the carbonate analysis, and additional mixtures, therefore, are required for replicate determinations. When values other than carbonate are intended, such as the content of exchangeable hydrogen and *p*H values upon the soil-calcite mixtures, the described standard technique is more advantageous.

The $Ba(OH)_2$–Ba-acetate titration procedure.—This procedure was designated and described as the "2-point titration" procedure (16). It prescribes preliminary suspension of 5 or 10 g of soil, in duplicate, in 50 ml of 0.5 *M* Ba-acetate at *p*H 7; the determination of the *p*H value of the suspension, and the computation of released hydrogen as read from a titration curve giving *p*H values against increments of acetic acid in 0.5 *M* Ba-acetate. Standard $Ba(OH)_2$ solution is then added to each system in different quantity, so one system will yield a *p*H value somewhat below 7 and the other, a value above 7. The quantity of $Ba(OH)_2$ required for titration of the systems to *p*H 7 is interpolated between the two points and expressed in m.e. per 100 g of soil. This procedure is less time-consuming than the one of serial titrations with $Ca(OH)_2$ in H_2O or $CaCl_2$ soil suspensions, in which procedure the base required at *p*H 7 is also obtained by interpolation between points above and below *p*H 7. Because of the variation in the soils' buffering properties, it is difficult to estimate the approximate base requirement to *p*H 7 from the preliminary *p*H reading in 0.5 *M* Ba-acetate. The frequent need for adjustment of such estimates to bring the system near *p*H 7 makes the procedure somewhat tedious and not suitable for routine operations.

RESULTS WITH REPLACEMENT METHODS AT *p*H 7

The data of Table 1 register the exchangeable hydrogen values that were obtained through 3 independent methods of replacement at *p*H 7 on

TABLE 1.—*pH values, exchangeable base content and exchangeable hydrogen of various types of soils and subsoils as determined through replacement with ammonium acetate, calcium acetate, and paranitrophenol buffer—all at pH 7*

SOILS				EXCHANGEABLE HYDROGEN WITH:			EXCHANGEABLE BASE—		
TYPE	SOURCE	PREDOMINANT COLLOID	pH	AM-ACETATE	CA-ACETATE	PARANITRO-PHENOL	CAPACITY[a] AT pH 7	CONTENT[b]	SATURATION
				m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*per cent*
Apison Silt Loam, 1940	Tenn.	Kaolinitic-Organic	6.0	2.1	2.2	1.5	5.2	3.0	58
Bolton Silt Loam, 1940	Tenn.	Kaolinitic-Organic	6.2	4.3	4.0	3.0	11.5	7.5	65
Canasuaga Silt Loam, 1940	Tenn.	Kaolinitic-Organic	6.0	2.9	3.3	2.0	11.3	8.0	71
Sequoia Silt Loam, 1940	Tenn.	Kaolinitic-Organic	5.3	6.0	4.9	3.2	7.4	3.2	44
Tellico Sandy Loam, 1940	Tenn.	Kaolinitic-Organic	5.6	5.9	5.4	3.6	11.7	6.3	44
Clarksville Silt Loam, 1947	Tenn.	Kaolinitic-Organic	5.5	3.3	4.1	3.0	6.7	2.6	40
Montevallo Silt Loam, 1940	Tenn.	Kaolinitic-Organic	5.0	4.9	5.0	3.7	7.7	2.7	35
Hartsells Sandy Loam, 1947	Tenn.	Kaolinitic-Organic	4.6	9.3	8.9	6.5	9.9	1.0	10
Claiborne Silt Loam, 1947	Tenn.	Kaolinitic-Organic	5.8	6.0	5.4	4.0	10.3	4.9	48
Cumberland Clay Subsoil, 1950	Tenn.	Kaolinitic	4.8	7.5	6.8	7.0	9.4	1.6	20
Dickson Silt Loam, 1940	Tenn.	Kaolinitic-Organic	4.9	3.9	3.6	3.0	5.0	1.3	26
Baxter Silt Loam, 1940	Tenn.	Kaolinitic-Organic	5.2	4.2	3.6	2.5	5.8	1.6	28
Dickson Silt Loam, 1950	Tenn.	Kaolinitic-Organic	4.9	2.4	3.1	—	7.5	4.4	59
Carrington Clay Subsoil	Ill.	Montmorillonitic	5.7	2.2	2.6	2.2	23.8	21.6	91
Talladega Clay Subsoil, 1940	N.C.	Kaolinitic-Organic	4.8	8.1	7.4	4.8	8.6	1.2	14
Miami Sandy Loam	R.I.		4.6	9.7	10.9	8.0	11.9	1.0	8
Wooster Silt Loam	Ohio		5.5	5.4	5.9	4.0	8.1	2.2	27
Onslow Sandy Loam, 1948	Va.	Organic	4.3	6.0	8.5	6.2	9.6	1.1	11
Portsmouth Fine Sand, Mucky Phase	Fla.	Organic	3.7	34.0	47.0	45.0	50.3	3.3	7
Volusia Silt Loam	N.Y.		5.5	5.4	5.5	3.5	10.7	5.2	49
Norfolk Sandy Loam, 1949	Miss.		4.9	2.8	3.0	2.4	3.7	0.7	20
Susquehanna Clay Subsoil, 1950	Ala.	Montmorillonitic	4.1	26.6	26.4	30.4	29.0	2.6	9

[a] The sum of exchangeable base content plus the exchangeable hydrogen thru calcium acetate replacement.
[b] Thru ammonium acetate replacement.

22 soils and subsoils. Related values, those for exchangeable base content, the computed exchange capacities and degrees of base saturation are given in the same table as background information. The Tennessee soils were chiefly from Knox and adjacent counties and characterized by the kaolinitic type of clay material and by low content of organic matter, and their exchange capacities ranged from 5 to 12 m.e. per 100 g. The soils and subsoils obtained outside of the State are characterized by high organic matter content or by the montmorillonitic type of clay. The selected soils offer a fairly complete representation of the principal types of colloids.

The results in the Ca-acetate and the ammonium acetate columns of Table 1, with exception of those from the two organic soils, show remarkable agreement between the exchangeable hydrogen values by the two procedures. The mean value of the 19 kaolinitic-organic soils in the ammonium acetate column is 5.3, whereas that in the Ca-acetate column is 5.6 m.e. per 100 g of soil. In the great majority of the soils tested the paranitrophenol buffer method gave results that were only $\frac{2}{3}$ to $\frac{3}{4}$ the values obtained by the other two methods, although in certain cases, as with the Cumberland clay subsoil and the Portsmouth fine sand, the comparisons show good agreement. On soils with high content of hydrogen the charge needs to be decreased to 2 g to give *p*H readings between 6 and 7, and consequently each 0.1 *p*H depression assumes the value of 5 m.e. of hydrogen per 100 g, instead of the 1 m.e. that is assigned to it when the soil charge is 10 g. Because of many deviations shown by it, the paranitrophenol method was not deemed sufficiently reliable for the correct evaluation of the content of exchangeable hydrogen in soils.

For determination of exchangeable hydrogen in soils, the Ca-acetate method offers the following advantages over the ammonium-acetate method: 1. The technique is less involved; 2. The procedure requires less time; 3. It is more precise; and 4. It is more adaptable for output in large number. Moreover, these advantages would hold even if a separate extraction with ammonium acetate were required to determine the replaceable cations (Ca, Mg, and K) on the same soils. In such case, the NH_4-acetate extraction could be simplified also, because no special set-ups are required for the careful washing of the NH_4-acetate. Large numbers of gravity filters can be set up readily and the cation extraction can be carried out in large numbers with but little attention or effort. Then too, when the determination of the adsorbed NH_4 is not contemplated, the NH_4-acetate solution would not require the precise adjustment to *p*H 7.

RESULTS OF HYDROGEN REPLACEMENT AT *p*H 8.1 OR ABOVE

The $CaCO_3$-equilibration proposal of Bradfield and Allison has been studied (1, 8, 10) not solely as a quantitative basis for a "base-saturated

soil" but also as a standard of comparison for various methods of determination of exchangeable hydrogen. Mehlich (8) obtained equally good results through the use of the much simpler procedure of triethanolamine-$BaCl_2$ buffer of *p*H 8.1. The results obtained by us on five distinct types of soils and subsoils by means of the two procedures are given in Table 2. In every instance the base-adsorption value by $Ca(OH)_2$-air equilibration method was from 30 to 50 per cent larger than that by the triethanolamine buffer. It is difficult to see in what particular our $Ca(OH)_2$-air equilibration treatment might have been too drastic, or in what particular the triethanolamine treatment might be inadequate, either of which might

TABLE 2.—*Exchangeable hydrogen content of various soils and subsoils, as determined through replacement by means of triethanolamine buffer, $Ba(OH)_2$ titration, $Ca(OH)_2$-air equilibration, and calcite decomposition—all at pH above 7*

SOILS		EXCHANGEABLE HYDROGEN THRU—				
		TRIETHANOLAMINE BUFFER	$Ca(OH)_2$-AIR EQUILIBRATION 48 HRS.	$Ba(OH)_2$ IN Ba-ACETATE TITRATION	CALCITE DECOMPOSITION	
TYPE AND SAMPLING DATE	SOURCE				24 HRS.	14 DAYS
		m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Hartsells Sandy Loam, 1947	Tenn.	13.0	16.7	8.5	9.3	12.5
Claiborne Silt Loam, 1940	Tenn.	10.2	14.4	5.6	6.0	8.1
Cumb. Clay Subsoil, 1949	Tenn.	11.2	—	—	—	—
Cumb. Clay Subsoil, 1950	Tenn.	10.0	15.1	7.2	8.1	10.0
Susquehanna Clay S.S., 1950	Ala.	25.0	32.8	25.0	28.3	29.2
Onslow Sandy Loam, 1948	Va.	10.0	13.2	7.7		11.3
Portsmouth Sandy Loam, 1940	N.C.	86.0		52.0	44.8	55.7
Volusia Silt Loam, 1915	N.Y.	8.2				5.4
Clarksville Silt Loam, 1947	Tenn.	6.4		4.3		6.0
Montevallo Silt Loam, 1940	Tenn.	7.6		4.4		5.8
Talladega Clay Loam, 1940	N.C.	12.5		7.3		9.6
Tellico Sandy Loam, 1940	Tenn.	12.0		7.8		7.4
Miami Sandy Loam,	R.I.			9.8		13.6

serve as explanation for the disparities between the results obtained by the means of the two methods. A parallel series of the $Ca(OH)_2$-air equilibration determinations was made for a 24-hour period, and the results were virtually identical with those given in Table 2.

The $Ba(OH)_2$ titration of soil suspensions in 0.5 *M* Ba-acetate on 6 representative soils and subsoils gave results which were almost identical with those obtained by means of the 0.5 *M* Ca-acetate replacement method given in Table 1. The $Ba(OH)_2$-titration method is more laborious

and offers no advantage over the simpler Ca-acetate replacement method that calls for only a single titrative determination.

The soil-calcite reaction progress for the 24-hour and 14-day periods are given in the last 2 columns of Table 2. During the shorter period the 5 soils and subsoils induced calcite decompositions in exact equivalence of the exchangeable hydrogen values that were determined by means of Ca-acetate replacement. During the following 13 days, the soil-calcite reaction diminished greatly, but progressed sufficiently so that the calcite decompositions in the 14-day period were nearly equivalent to the findings for exchangeable hydrogen as obtained by means of the triethanolamine buffer procedure. The calcite reaction values were still considerably below the values obtained by the $Ca(OH)_2$-air equilibration procedure. The probability of attainment of equilibrium values in the soil-calcite reaction will be considered further in the following paragraphs.

INVESTIGATION OF SOIL-CALCITE REACTION—FACTORS THAT AFFECT RATE OF REACTION

The principal objective of the present study was to determine the conditions that induce speediest soil-calcite reaction in soils of representative types, and, if possible, to ascertain the time required to obtain equilibrium values. In general, the factors that affect the rate of soil-calcite reaction may be recognized in advance, but their relative importance can be established only through experiment. Consideration was given to the following variables:

(a) The soil: its predominant type of colloid, its cation exchange capacity, and its degree of unsaturation.
(b) The carbonate material: its purity, fineness, and rate of addition.
(c) The average moisture content of the system and the moisture cycle.
(d) The temperature of the soil-calcite system.
(e) The time interval.

The soil factor.—The following soils were selected as representative of kaolinitic, montmorillonitic, and organic colloids, and of colloidal materials devoid of organic matter:

Hartsells sandy loam, kaolinitic-organic colloids.
Claiborne silt loam, kaolinitic-organic colloids.
Cumberland clay subsoil, kaolinitic colloids, practically devoid of O.M.
Susquehanna clay subsoil, montmorillonitic, practically devoid of O.M.
Portsmouth fine sand, organic, practically devoid of inorganic colloids.

The exchangeable hydrogen contents of these soils by the Ca-acetate replacement method ranged from 6 m.e. to 47 m.e. per 100 grams of air-dry material. This wide range of exchangeable hydrogen values among the selected soils should provide results of broad usefulness. The Susque-

hanna subsoil was not available for inclusion in the early studies of moisture and temperature effects.

Carbonate, and rates of incorporation.—Iceland Spar of 325-mesh was used in preference to precipitated $CaCO_3$ to ensure against impurities that might exert an adverse effect upon the reaction, especially when additions are made at high rates. The first 3 soils received 1 g of calcite, or 20 m.e. per 100 g of soil, whereas the other 2 soils received inputs equivalent to 40 and 70 m.e., respectively. Calcite incorporations were calculated to provide a good excess at the end of the reaction period.

TABLE 3.—*The effect of moisture content upon the speed of soil-calcite reaction at 30°C.*

SOILS	325-MESH CALCITE ADDED PER 100 g	DURATION OF CONTACT	CALCITE DECOMPOSITION PER 100 g AT PERCENTAGES OF MOISTURE SATURATION		
			60	80	100
	m.e.	*days*	*m.e.*	*m.e.*	*m.e.*
Hartsells, 1947	20	5	11.2	10.6	10.2
	20	10	12.0	11.6	10.9
	20	15	12.6	12.2	11.6
Claiborne, 1940	20	5	7.6	7.2	5.2
	20	10	8.6	8.6	7.1
	20	15	9.3	9.0	7.8
Cumb. Subsoil, 1949	20	5	10.8	10.8	10.4
	20	10	11.2	11.4	11.1
	20	15	11.7	11.6	11.4
Portsmouth Muck, 1940	70	5	57.0	57.4	58.1
	70	10	60.6	61.5	59.2
	70	15	63.4	62.8	63.6

Large excesses—above 15 m.e.—were avoided because large residues of $CaCO_3$ would entail correction for the dilution effect of the added calcite upon the soil charge.

Moisture as a reaction variable.—In many of the earlier investigations the soils were wetted slowly until the moisture just penetrated to the bottom of the beaker. This provided a moisture content of close to 80 per cent of the water-holding capacity of the soils. To determine moisture content best suited for the soil-calcite reaction, a number of soils were mixed with the requisite quantities of 325-mesh calcite and wetted to saturation levels of 60, 80, and 100 per cent. The wet mixtures were kept at 30°C. for respective periods of 5, 10, and 15 days. The mixtures then were dried and analyzed for residual $CaCO_3$. The computed calcite decompositions

are given in Table 3. The results from the Cumberland subsoil and the Portsmouth fine sand show little difference in the speed of calcite decomposition that could be attributed to moisture variation within the range of 60 to 100 per cent saturation. On the other hand, the Hartsells and the Claiborne soils show small yet significant decrease in calcite decompositions at the 80 per cent saturation, and a greater decrease at the 100 per cent level, as against decompositions at the 60 per cent level. Upon basis of the results of Table 3, it appears that the reaction progress is equally as good at the 80 as at the 60 and 100 per cent moisture levels in 2 soils. On the other two soils, however, the reaction was significantly more advanced at the 80 per cent level than the 100 per cent moisture level, yet only slightly less than the extent shown for the 60 per cent level. Because of these observations, and due to the fact that with the 60 per cent moisture saturation the bottom portion of the soil remained unwetted for hours, it was concluded that for purposes of Ca-saturation by the means of 325-mesh calcite, the 80 per cent moisture saturation would prove the most satisfactory moisture level. It should be noted, however, that this conclusion as to best moisture condition will not apply to the soil-calcite reaction in general. Experiments with calcite particles of varying sizes, which are not given here, have shown that the greatest speed of reaction with coarser calcite separates is attained at the higher moisture levels, 90–100 per cent saturation. The superiority of the higher moisture levels is more evident with the coarser calcite particles and shorter duration of contact.

Effect of temperature.—Rise in temperature causes an increase in the chemical activity of the reacting materials through acceleration of the diffusion of solute Ca from points of high concentration to zones of lower concentration. Rise in temperature also decreases the concentration of CO_2 in the solution phase, thus raising the *p*H of the solution and tending towards greater 'Ca-sorption' in the soil. Hence we may expect that differences in temperatures will induce differences in speed of reaction as well as in equilibrium values.

Several soils and one subsoil were brought to 80 per cent moisture saturation and exposed to constant temperatures of 10°C. and 30°C. for various periods of from 5 to 90 days. One soil also was subjected to a temperature of 20°C. Resultant calcite decompositions, or Ca-sorption values, in m.e. per 100 g of soil, are given in Table 4. For the five-day period, differences between the calcite decompositions at 10°C. and 30°C. were only 1.3 m.e. on the Cumberland clay subsoil, 2 m.e. on the Hartsells sandy loam and 3.2 m.e. on the Portsmouth fine sand of high organic matter content. Time progression registered no effect on the temperature differential of the Cumberland subsoil, for which the average value was only 1.5 m.e. for the 10, 15, and 30-day periods; the reaction progress after the 10-day period was meager and appeared at a standstill

between the 10 and 30-day periods. The temperature differential on the Hartsells soil was 2.0 m.e. for the first 15 days, 3 m.e. and 4.1 m.e. for the 30 and 90-day periods, respectively. Calcite decompositions in the Hartsells soil showed continued progress at both 10°C. and 30°C. for the entire time range, but greater progress was attained at 30° C. These observations apply alike to the Claiborne soil, as can be seen from the comparisons in Table 4.

TABLE 4.—*Calcite decomposition by soils and subsoils at 10° and at 30°C., with 80 per cent moisture saturation*

SOILS	325-MESH CALCITE ADDED	TEMP. OF SYSTEM	CALCITE DECOMPOSITIONS PER 100 g DRY SOILS AT CONTACT PERIODS IN DAYS:					5-DAY AS FRACTION OF 90-DAY DECOMPOSITION
			5	10	15	30	90	
	m.e.	°C.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*per cent*
Hartsells, 1947	20	10	8.7	9.4	9.3	10.1	10.7	81
	20	30	10.7	11.7	12.2	13.1	14.8	72
Claiborne, 1940	20	10	—	—	—	7.0	7.8	—
	20	30	7.3	8.6	9.2	9.8	10.9	67
Cumberland Subs, 1949	20	10	9.5	9.8	10.2	9.7	10.0	95
	20	30	10.8	11.4	11.6	11.2	12.0	90
Portsmouth Muck, 1940	70	10	54.3	57.2	58.6	63.5	67.1	81
	70	30	57.5	61.5	62.8	66.4	67.5	85
	100–200							
Hartsells, 1940	20	10	—	5.2	—	5.7	6.7	
	20	20	—	—	—	7.3	7.9	
	20	30	—	5.7	—	8.2	9.0	

The Portsmouth soil showed a constant differential for carbonate decomposition as to temperature effect for the periods up to 15 days, but registered a sharp decrease in such differential for the 30 and 90-day periods. This may be due to the depletion of the added calcite and consequential stoppage in the reaction-progress at both the 10°C. and the 30°C. temperatures at some point between the 30 and 90-day periods. More conclusive results probably could have been obtained on this soil had it been supplied with 80 or 90 m.e. of calcite per 100 g, instead of the 70 m.e. input that was insufficient to satisfy the soil's Ca-sorption capacity.

From the limited data of Table 4 it may be concluded that there is a constant difference in calcite decomposition by surface soils at 10°C. and 30°C. temperatures for early periods up to 15 days, and that the difference widens because of the comparatively greater reaction progress at 30°C. after 15 days. A part of the greater reactivity at 30°C. is probably due to

TABLE 5.—*Progressive decomposition of calcite by acidic soils and subsoil in moist contact at 30°C*
Long Range Series

DURATION OF CONTACT	RESIDUAL CARBONATE DETERMINATIONS				CARBONATE DECOMPOSITION		RESIDUAL CARBONATE DETERMINATIONS				CARBONATE DECOMPOSITION	
	1	2	3	AVG.	PER PERIOD	AVG. INCREMENT PER 5 DAY PERIOD	1	2	3	AVG.	PER PERIOD	AVG. INCREMENT PER 5 DAY PERIOD
Days	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
	Hartsells Sandy Loam, 1947						Cumberland Clay Subsoil, 1949					
5	9.4	9.3	9.3	9.3	10.7	10.7	9.3	9.1	9.2	9.2	10.8	10.8
10	8.5	8.2	8.3	8.3	11.7	1.0	8.4	8.7	8.6	8.6	11.4	0.6
15	8.0	7.7	7.7	7.8	12.2	0.5	8.3	8.4	8.4	8.4	11.6	0.2
30	6.9	6.9	6.9	6.9	13.1	0.3	8.7	8.8	8.8	8.8	11.2	—
90	5.2	5.3	5.1	5.2	14.8	0.14	8.0	7.9	8.1	8.0	12.0	0.07
180	4.4	4.6	4.4	4.5	15.5	0.04	6.9	7.1	7.3	7.1	12.9	0.05
	Claiborne Silt Loam, 1940						Portsmouth Sandy Loam, 1940					
5	12.6	12.9	12.5	12.7	7.3	7.3	12.7	12.1	12.6	12.5	57.5	57.50
10	11.3	11.5	11.4	11.4	8.6	1.3	8.4	8.5	8.6	8.5	61.5	4.00
15	10.9	11.0	10.5	10.8	9.2	0.6	7.2	7.2	7.1	7.2	62.8	1.30
30	10.2	10.2	10.2	10.2	9.8	0.2	4.0	3.5	3.5	3.6	66.4	1.20
90	9.1	9.2	9.0	9.1	10.9	0.09	2.5	2.4	3.2	2.5	67.5	0.90
180	8.3	8.4	8.2	8.3	11.7	0.04	0.6	0.6	0.6	0.6	69.4	0.05

the greater biochemical activity of the soil at the higher temperature; in particular, increased nitrification. The influence of additional factors that may be responsible for the sudden spurt in calcite decomposition beyond the 15-day period needs to be determined through more extensive experiments. In contrast to the surface soils, the Cumberland clay subsoil continued to show a constant differential for temperature effect in its reactivities between 10°C. and 30°C. up to the 30 day, and possibly through the 90-day period. Because facilities for maintenance of constant temperatures of 10°C. and 20°C. were not readily available, the subsequent studies on the soil-calcite reaction were conducted at 30°C.

Soil-calcite reaction progress at 30°C.—Long-time experiments of calcite decomposition were inaugurated on typical soils with sufficient replicates so that the analyses for residual carbonate after various contact periods of from 5 days to 1 year could be made as on a series of independent experiments. The results, other than those for the concluding 6 months, are given in Table 5 and are plotted in Figure 2. Plotted also are the decompositions effected by the Susquehanna clay subsoil for periods of from 1 day to 21 days. Because the values for the Susquehanna subsoil and those for the Portsmouth soil are plotted at one-tenth proportion, their plotted values should be multiplied by 10 to obtain actual values. The data of Table 5 and the plotted values in Figure 2 serve to indicate whether the equilibrium of calcium saturation can be attained when a soil is left in contact with an excess of finely divided calcite for various periods up to 180 days. The two clay subsoils were used to ascertain characteristic reaction progress for the kaolinitic and the montmorillonitic types of clays, as distinguished from soils of kaolinitic-organic nature, such as the Hartsells and Claiborne soils. The two last-mentioned soils reacted with calcite very rapidly during the first 15 days and the reactions continued throughout the remainder of the 180-day period. After 15 days, and up to 180 days, the increase in calcite decomposition for the 2 soils was about 25 per cent of the decomposition that occurred during the initial 15 days of contact. The Portsmouth fine sand (highly organic) behaved likewise but the 70 m.e. calcite had been decomposed completely before the end of the 180-day period.

The Susquehanna clay subsoil, a predominantly montmorillonitic clay, attained apparent saturation at the 7-day contact period, 93 per cent of the calcite decomposition having occurred during the first day of contact. No such relationship can be computed for the other soils since none of those attained equilibrium saturation within the 180-day experimental period. The graph of Figure 2 shows that experimental data from the kaolinitic Cumberland subsoil do not lie on a continuous curve; the 3 points of the 5, 10, and 15-day periods lie considerably above the position of the curve formed by the results of the 30, 60, and 90-day periods. The cause of this discrepancy is not known, but it is possible that two

different batches of the same type of clay, with different hydrogen contents, were used in the two experimental groups. The fact remains that the decomposition of calcite by the kaolinitic clay subsoil progressed 0.8 m.e. for the periods from 30 to 90 days, and 0.9 m.e. in the 90 to 180-day period. These increases in calcite decomposition are slightly less, but of same order of increase as that effected by the Hartsells and Claiborne soils during corresponding contact periods.

The foregoing findings demonstrate that soils of both the kaolinitic-organic and the kaolinitic-only adsorption complexes continue to decompose calcite during a period of 180 days, although continually at decreasing rate of speed. It cannot be predicted when this calcite decomposition will terminate or what its value then might be. Only the montmorillonitic Susquehanna subsoil appears to have come to equilibrium with calcite in the brief period of 3 to 7 days and apparently remained in that status to the end of the 21-day experimental period. The decreasing speed of the soil-calcite reaction in the present experiments can be judged from the 5-day average increments of the calcite decompositions for the successive time intervals, as shown for each soil in the right-hand columns in Table 5. After the first 30 days, the increases in the calcite decompositions registered through a sequence of determinations at 5-day intervals would be so small as to give the appearance of equilibrium status. Positive reliance as to equilibrium values may be placed only upon cumulative results from long-range experiments on soil-calcite decompositions.

The continuous decomposition of calcite by soils in the present experiment serves to confirm, in a general way, the results obtained in experiments on the decomposition of additive carbonates in soils that were conducted in this laboratory in 1913 (4). For more graphic comparison of relative speeds, the CO_2-evolution data from the older experiment on a "clay subsoil" were plotted and traced in Figure 2. The relative speed of the soil-carbonate reactions in the earlier and present experiments need not be based on comparable evolutions of CO_2 at a particular period, but rather on their respective progress during 30-day periods in relation to their corresponding values for intervals of 180 days. In the earlier experiment the evolution of CO_2 during the 30-day period was 75 per cent of the evolution that occurred in 180 days, as against a corresponding proportion of 90 per cent in the present experiment. These comparisons indicate that, under the conditions of the present experiments, the carbonate decompositions were proceeding at a speedier rate.

SOIL-CALCITE REACTIONS IN COMPARISON WITH EXCHANGEABLE HYDROGEN VALUES

The data from the preceding tables are summarized in Table 6 to show the calcite decomposition values on 5 soils for the periods of 1 day and of 14 and 180 days, along with corresponding values for exchangeable hy-

drogen. With one exception, the 180-day decompositions exceeded the values obtained for exchangeable hydrogen values by means of the Ca-acetate and triethanolamine methods. The triethanolamine gave much higher value for the Portsmouth fine sand. The capacity of this soil to effect calcite decomposition at 180 days could not be determined because of the decomposition of the entire addition of calcite. The $Ca(OH)_2$-air equilibration for 48 hours gave values higher than those derived from the 180-day contact between soil and calcite. The greatest differences are those shown for the two subsoils. It is surprising that the Susquehanna clay subsoil should indicate additional Ca-sorption from the $Ca(OH)_2$-air system, since that clay has shown a stable value for calcite decomposition.

TABLE 6.—*Comparison of calcite decomposition values with exchangeable hydrogen values obtained by representative procedures*

SOIL AND SUBSOIL	CALCITE DECOMPOSITIONS PER 100 g. BY PERIODS IN DAYS			EXCHANGEABLE HYDROGEN BY THE SEVERAL PROCEDURES		
	1	14	180	Ca-ACETATE	TRIETHANOLAMINE	$Ca(OH)_2$-AIR EQUILIBRATION
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Hartsells Sandy Loam	9.3	12.5	15.5	8.9	13.0	16.7
Claiborne Silt Loam	6.0	8.1	11.7	5.4	10.2	14.4
Cumb. Clay Subsoil	8.1	10.0	12.9	6.8	10.0	15.1
Susquehanna Clay Subsoil	28.3	29.2	—	26.4	25.0	32.8
Portsmouth Fine Sand	44.8	55.7	63.0	47.0	86.0	—

Moreover, because of the slow reaction progress shown by the kaolinitic clay subsoil at the 180-day contact period, it is doubtful that the calcite decomposition would ever attain a value equal to the one obtained for that clay by means of the $Ca(OH)_2$-air equilibration procedure. Because of the indeterminate nature of the calcite decomposition through natural contact with soils, as shown by the present experiments, the theoretically defined standard for a "base-saturated soil" (1 p. 64) is deemed to be without basis of reality, and the postulation as to equilibrium values is without counterpart under natural conditions.

It appears that, for the present, the adoption of the Ca-acetate replacement method at *p*H 7, with the potentiometric titration of the replaced hydrogen at *p*H 8.8, would provide a simple and precise procedure for determination of the lime requirement of soils.

SUMMARY

Several procedures for the determination of exchangeable hydrogen in soils were compared, as to precision and simplicity of technique, with the values obtained by carbonate decomposition as derived from a study of

mixtures of representative soils and excesses of finely divided calcite, during periods of from 1 day to 180 days.

The objectives were (a) to compare the relative merits of the procedures of each functional group and to establish the simplest and most precise procedure for each group, and (b) to compare results on exchangeable hydrogen by the various procedures, with the values derived by the decomposition of added calcite. The authors sought to evaluate the usefulness of the various procedures, either to define the theoretical "base-saturated soil" or to supply information on the lime requirement necessary to raise the *p*H values of various soil types to neutrality.

The procedures investigated were: (a) ammonium acetate, (b) calcium acetate, (c) paranitrophenol buffer, (d) $Ba(OH)_2$ titration of soil suspension in 0.5 *M* Ba-acetate to *p*H 7, (e) replacement of electrolytes at *p*H 8.1 or higher, (f) $Ca(OH)_2$-air equilibration, (g) triethanolamine-$BaCl_2$ buffer, and (h) decomposition of calcite in soils for periods of 1 day to 180 days.

Based upon comparative values from 22 soils, the NH_4-acetate, Ca-acetate, and paranitrophenol procedures gave results as follows: the paranitrophenol procedure gave only $\frac{2}{3}$ to $\frac{3}{4}$ the values obtained by either of the other two procedures on a large percentage of the soils used. Other than in few cases, the Ca and NH_4-acetate procedures gave concordant results, but the Ca-acetate procedure was deemed more precise and simpler for determinations in large numbers.

The $Ba(OH)_2$ titration of soil suspension in Ba-acetate to *p*H 7 gave results which, in most part, were in accord with those registered by the simpler Ca-acetate method. The results by triethanolamine procedure were from 50 to 100 per cent higher than those obtained by the Ca-acetate method on the kaolinitic-organic soils, whereas the two methods gave concordant values for the Susquehanna (montmorillonitic) clay subsoil.

The results by the $Ca(OH)_2$-air equilibration method on 5 soils were from 20 to 50 per cent higher than those obtained by the triethanolamine method. The results by the $Ca(OH)_2$-air equilibration method were also higher than the results obtained by calcite decomposition during 180 days at 30°C.

The various factors that effect the progress of soil-calcite reactions were investigated to establish the most favorable conditions. For purposes of Ca-saturation of soils, the following conditions were imposed: (a) calcite of 325-mesh in excess of from 5 to 15 m.e. over the probable Ca-sorption per 100 g of soil, (b) maintenance of moisture near 80 per cent of H_2O capacity, and (c) temperature of 30°C.

The results on calcite decompositions for various periods of from 1 day to 6 months indicate that the reaction with kaolinitic and organic soils was continuous during the entire 6-month period at a diminishing rate of speed, but without indication of attainment of equilibrium, whereas the

decomposition of calcite in the montmorillonitic subsoil registered equilibrium after 3 to 7 days of contact. Aside from the possibility of obtaining a standard "base-saturated soil" through equilibration in a mixture of soil and calcite, the soil-carbonate reaction studies are useful in the determination of the rates of reactivities between soil and limestone of different fineness and limestones of different chemical properties.

The results of the soil-calcite reaction studies indicate that none of the methods investigated serves to establish quantitative values for the ideally defined standard "base-saturated soil." The Ca-acetate replacement procedure at *p*H 7, with the titration of the replaced hydrogen to *p*H 8.8, was found to be the one best adapted for routine determination of the lime requirement necessary to raise the *p*H value of a soil to near neutrality. The experimental work on soil-calcite reaction rates is being continued with typical soils.

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BOOK REVIEWS

Soil Fertility and Sewage, J. P. J. VAN BUREN. Dover Publications, Inc. New York, N. Y., Bloemfontein, South Africa, 1948. 10+236 pages.

The author dedicates his book "to the first ten inches of soil." This gives an idea of the broad approach the author takes in his treatment of composting and other means of utilizing varied kinds of organic matter for soil improvement. Sanitary features are considered with respect to utilization of human wastes in varied ways. It is not at all likely that the citizens of the United States will very soon be willing to follow the lead of the South Africans with respect to utilizing human wastes in composts. Many useful suggestions are included, however, regarding other means of conserving such materials.

Anyone undertaking a study of composts could well consider the material presented in a chapter dealing with general principles governing the process of composting. The book describes in detail various equipment that has been designed for composting. An adequate number of photographs illustrate the text. Literature cited includes references from different parts of the world.

S. M. ANDERSON

Organophosphorus Compounds, By GENNADY M. KOSOLAPOFF, John Wiley & Sons, Inc., 440 Fourth Ave., New York 16, N. Y. 1950. 376 pages. $7.50.

This is a highly useful book for the organic chemist who would like to know more about the peculiar reactions and properties of organic phosphorus compounds. In recent years a great deal of practical interest has developed in this little known branch of organic chemistry. The author, a prominent investigator in this realm, points out in the preface that the only other treatise on organophosphorus chemistry is that of V. M. Plets published in Russian in 1938 in the U.S.S.R.

In a brief introduction the author sketches the highlights of the history of organophosphorus chemistry, singling out some of the outstanding contributors and the foundations they laid. There follows an outline of the scope and plan of his book. The author closes his introduction with some important practical hints and cautions, on laboratory work with phosphorus compounds.

One of the most commendable features of Kosolapoff's comprehensive work is his neat avoidance of nomenclature difficulties by a systematic use of structural line formulas. About 4000 organophosphorus compounds are listed with their salient physical properties, and references to their preparation. The compounds have been divided and classified according to an arbitrary but practical plan. Starting with phosphines, the author proceeds systematically to halophosphines; halophosphine halides and phosphonyl halides; quaternary phosphonium compounds; tertiary phosphine oxides, sulfides and selenides; phosphinous, phosphonous, and phosphonic acids, their sulfur analogs and esters; phosphites and thiophosphites; phosphates, halophosphates, and thio analogs; compounds with phosphorus to nitrogen bonds; quasi-phosphonium compounds; derivatives of anhydro acids. There is an appendix to summarize new chemical information that accumulated in the literature in 1949 during the compilation of the main body of the book. The book has a subject index.

In each chapter the various methods of preparation are described first, each method being numbered with a Roman numeral. Next the general characteristics are given. Then the individual compounds are listed by line structure, with Roman numeral corresponding to the method of preparation. Salient physical properties of each compound are given with arabic number superscripts to tie the compound to

the list of references which follows and closes each chapter. The references are as nearly complete as possible. The scheme is an excellent one for saving the chemist's time in tracking down the available information on a particular compound or class of compounds without having to think of the various ways in which the compound might be named. Since organophosphorus nomenclature is in a particularly chaotic state, it is a great advantage to have a book that is nearly independent of naming difficulties.

Dr. Kosolapoff is to be commended for his systematic and painstaking work in producing this book. It will be of use and interest to every organic chemist who has any occasion at all to deal with phosphorus compounds.

S. A. HALL

Soybeans and Soybean Products, Vol. I. Klare S. Markley, Editor, Interscience Publishers, Inc., 250 Fifth Ave., N. Y. 1, N. Y. 1950. xvi+540 pages, $11.00.

The following Publisher's Note appears following the Preface:

"The original plan for *Soybeans and Soybean Products* was for one volume, but the vastness of the subject and the extensive illustrations made a single volume so cumbersome that it was found desirable to create two volumes of equal size. The author and subject indexes will be found at the end of Volume II. A complete list of contributors is given on pages vii–viii following. The detailed contents of the first volume is followed on page xvi by a list of chapter titles for the second, to give the reader the complete scope of this work. The volumes are paginated through to avoid any confusion in indexing and citation of sources."

The subject matter is divided, more or less logically, into four broad sections: Production, Structure and Composition, Processing, and Utilization. Volume II will deal with Utilization only, except that it will include a final chapter under Processing called "Solvent Extraction Processes." It will also carry an author index and a subject index for both volumes. The price of Vol. II will also be $11.00.

Each of the chapters in Vol. I has been written by a specialist in the particular field covered. A fair idea of the broad scope, comprehensive coverage, and authoritative character of the volume may be gained from a mere listing of the chapter titles and authors. A brief explanatory comment has been added on chapters of special reader interest. The list follows:

History of Soybean Production—W. J. Morse.

World Soybean Production and Trade—E. L. Burtis.

Structure and Genetic Characteristics of the Soybean—L. F. Williams.
This chapter deals with all parts of the plant: seed, root, stem, leaf, flower, and pod.

Chemical Composition of Soybean Seed—W. J. Morse.
Every conceivable angle of chemical composition is fully covered.
There are 13 tabulations and 73 references.

Chemical Composition of Soybean Oil—B. F. Daubert.
What was said of the previous chapter is equally applicable here.
The topic is exhaustively treated, with 17 tabulations and 173 references.

Chemical Characteristics of Soybean Oil—Stewart T. Bauer.
Methods of determining the chemical and physical characteristics of oils are given along with variation and significance of the numerical values. There are 77 references.

Physical Properties of Soybean Oil—M. E. Jefferson.

Proteins and Other Nitrogenous Constituents—Sidney J. Circle.
Among the broad topics included are soybean meal, isolation of protein, chemical and physical properties of the proteins, hydrolysis of the proteins, etc.

Other Constituents of the Soybean—B. F. Daubert.
Various carbohydrates and organic phosphorus compounds make up the bulk of this chapter.

Nutritive Factors in Soybean Products—H. H. Mitchell.
Such topics are included as available energy and protein, minerals, vitamins and co-nutrilites.
Grading and Evaluation of Soybeans and Derived Products—Egbert Freyer.
This initial chapter under "Processing" covers sampling, grading, standards for meal and flour and oil.
Handling and Storage of Soybeans—Leo E. Holman.
Biological Processes in Stored Soybeans—Max Milner.
Mechanical Processing of Soybeans—Louis F. Langhurst.

The wealth of factual material will make the book valuable both to the regulatory official and to the investigator. References to each chapter are abundant and up-to-date. The cuts, graphs, and tabulations are numerous and well selected and presented. The format is attractive and the editing appears to be excellent.

Volume II will contain the final chapter of the "Processing" section and the following ten chapters under "Utilization":

Production and Utilization of Lecithin—Joseph Stanley.
Processing of Edible Soybean Oil—J. W. Bodman, E. M. James, and S. J. Rini.
Nutritional Value of Soybeans and Soybean Products—Harry J. Deuel, Jr.
Edible Soybean Oil Products—H. C. Black and K. F. Mattil.
Soybean Oil By-Products—R. W. Lehman and N. D. Embree.
Nonedible Soybean Oil Products—Theodore F. Bradley.
Soybean Oil Meal for Livestock and Poultry—J. W. Hayward.
Soybean Protein Food Products—R. S. Burnett.
Soybean Protein Industrial Products—R. S. Burnett.
Other Soybean Products—Janice M. Smith and Frances O. Van Duyne.

V. E. MUNSEY

The Smut Fungi, A Guide to the Literature, with Bibliography. By GEORGE W. FISCHER. The Ronald Press Co., New York, N. Y., 387 pp. 1951.

As in most sectors of the biological field there have been extensive accumulations of data on the smut fungi and the very important plant diseases, particularly of our cereal crops, which they cause. The literature on the subject is scattered through a bewildering array of journals, proceedings, reports, bulletins, and even entire books published in many languages in most of the countries of the world over a period of more than 100 years.

The author of the book under review has devoted many years to the study of the smut fungi and is well qualified for the task of bringing order out of the chaos suggested by 3353 literature references which constitute slightly more than two-fifths of his book. Preceding this bibliography he has presented a subject matter guide, based on these references, to the control and biology of 330 species of the *Ustilaginales*, which are arranged in alphabetical order under their genera, 25 in number. Fairly complete synonymy is cited for each species, a useful and rather necessary feature, if one is to delve successfully into all that is known about these fungi, since many of them have paraded under many an alias or synonym. The subject matter for each species as derived from the references is presented chronologically under the headings, Control; Culture on Artificial Media; Cytology; Heterothallism and Sex; Hybridization and Genetics; Life History, and Parasitism; Longevity of Spores; Miscellaneous Records; Physiologic Specialization; Spore Germination and Varietal Resistance and Susceptibility. Taxonomy and geographical distribution have not been considered, since it was the author's expectation that George L. Zundel's work on The Ustilaginales of the World covering these points would be published in advance of his own book. Most unfortunately it does not appear probable at this time that the Zundel manuscript will ever be published.

JOHN A. STEVENSON

Advanced Organic Chemistry, Reynold C. Fuson, John Wiley & Sons, Inc., New York, N. Y. 1950. x+669 pages. Price $8.00.

For a profitable use of "Advanced Organic Chemistry" the reader needs a background in the subject. Electronic concepts are interwoven into those organic reactions where such ideas are helpful, but the equations are in the form well known to all organic chemists. The physico-chemical treatment is not overdone.

Perhaps the outstanding feature of this book is its arrangement of subject material—a distinct change from the usual textbook treatment. A second noteworthy attribute is the attention to the practicality of the reactions which are discussed. Most organic chemists who have had research experience with synthetic or degradative problems recognize that many organic reactions are only "paper" reactions as far as their usefulness for preparative purposes is concerned. The author has made a special effort to point out how practical a reaction is by giving the yield that is reported in Organic Syntheses or in some other reliable source. The book is up to date, as some 1950 references show.

The contents of this book are not all-inclusive, as no single volume can be in a subject as big as organic chemistry; its 25 chapters include in addition to many expected subjects, such topics as: Cleavage of Carbon-Carbon Bonds; Aliphatic Substitution; Halogen Compounds; Organometallic Compounds in Synthesis; Carbon Monoxide in Synthesis; Oxidation; Acylation of Aromatic Compounds; Ring Closures Involving Condensation of Carbonyl Groups with Aromatic Nuclei; Active Methylene Compounds; Conjugate Addition; Aromatic Character and Polymerization.

This volume is not intended to be a reference book. The heterocyclic compounds are hardly mentioned, except as their formation may illustrate a reaction. Some of the limitations of many reactions are discussed; this is a desirable trend. For the reader who desires to know more than the usual elementary text tells about organic chemistry, this new volume will be very helpful.

W. I. PATTERSON