WEDNESDAY

REPORT ON NAVAL STORES

By V. E. GROTLISCH (Office of Distribution, War Food Administration, Washington, D. C.), *Referee*

No collaborative work on naval stores has been undertaken by the Referee on Naval Stores. However, your Referee is also Chairman of the Committee on Naval Stores of the American Society For Testing Materials, and in that capacity has for some years been giving consideration to a number of matters in connection with the preparation and revision of analytical test procedures applicable to both turpentine and rosin, as well as to a number of other naval stores items, such as pine oil, pine tar, etc. Some of these may be of interest to A.O.A.C. However, it is felt that until the methods of analysis for such other naval stores products have enjoyed tentative standard status in A.S.T.M. for at least a year, it is not advisable to offer them for consideration by A.O.A.C. at this time.

The chapter on Naval Stores of the *A.O.A.C. Methods of Analysis*, covering turpentine and rosin, has been carefully reviewed in order to clarify and shorten the language where possible, and to determine what recommendations could be made at this meeting, in the light of our past experience and the standards adopted by A.S.T.M. Recommendations are offered which will be incorporated in the 6th edition of the *Methods of Analysis*.*

The Referee doubts the need for inclusion of the two sections on rosin grading (9 and 10) in the *A.O.A.C. Methods of Analysis*. It is questionable whether these are of any interest to members of the Association who use the book, and it does not appear that the value of the book would be lessened if these sections are omitted. There is even some question whether any of the rosin methods are of much interest to official agricultural chemists; but since these methods do not take up much space they could be included in the Book for the sake of completeness and as a source of information on the subject.

No report on radioactivity was given by the Referee.

No report on Quantum Counter was given by the Associate Referee.

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* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 46, 73 (1945). The changes recommended and details of the methods will be incorporated in the revision of the *Methods of Analysis*, 6th edition, 1945.

REPORT ON ANALYSIS BY RADON MEASUREMENT AND ALPHA PARTICLE COUNTING

By FRANCIS J. DAVIS (National Bureau of Standards, Washington, D. C.), *Associate Referee*

The problem of measuring quantities of radium can be divided into groups depending primarily on the quantity to be measured: (1) Large quantities of the order of milligrams, using the electroscope for measurement; (2) intermediate quantities of the order of micrograms, using the Geiger counter, and (3) small quantities below 10^{-8} grams Ra, using the emanation, or radon method.

(1) *The electroscope*, either the gold leaf type, or the Wulf string type, still remains the best instrument for measurements of relatively large quantities of radium. The gold leaf electroscope is excellent for quantities over one milligram of radium, while the Wulf string type, as described in *Methods of Analysis, A.O.A.C.*, 557 (1940), is very useful for quantities of over 10^{-6} grams.

(2) *The Geiger-Müller counter* is a simple device consisting essentially of an outer cylindrical electrode and a central wire in a partial vacuum and a gas mixture of usually a rare gas, such as argon, and an organic vapor, such as alcohol. The electrode may be completely enclosed in a glass envelope as is usually the case in a gamma ray counter, or the cylindrical electrode may be sealed in glass only at the ends, such as in a beta-ray counter, where the outer electrode must have a minimum of absorption for beta rays and is usually of thin-walled aluminum with a thickness of the order of 0.005 inch. Some beta-ray counters are made with thin glass walls and a layer of silver deposited on the inside for the outer electrode; others are made with a mica, or thin glass window on the end, or side. The Geiger-Müller counter requires a high voltage supply of about 1,500 volts, as well as an electronic scaling circuit and a recorder such as a telephone message register. The circuit may be constructed for use with several different Geiger-Müller counters, which may be simply substituted one for the other. The counter tube in the apparatus constructed by the National Bureau of Standards for the radium laboratory of the Food and Drug Administration had the dimensions of the outer electrode 3.5×22 cm. The sensitivity of this counter to 100 micrograms of radium at 1 meter was about 30 counts per second above background, the background rate being about 5 per second. The sensitivity to 0.1 μgm placed as close as possible is about 5 net count/sec.

The background rate and sensitivity of the Geiger-Müller counter should remain approximately the same and should be checked each time it is used to make certain that it is operating properly.

In measurements of samples by either electroscope or Geiger-Müller counters we must be concerned with absorption of the radiation in the

sample itself. In some cases the absorption can be estimated quite easily, in others it must be measured. Franz and Weiss[1] have described a method used with a gamma ray ionization chamber to determine the absorption, and by the help of an experimental relation between density and absorption they made radium determinations within 2 per cent.

The Geiger-Müller counter was adopted by the A.O.A.C. as official, first action, in 1942. If the Geiger counter is used as suggested in *This Journal*, 25, 103 (1942), the accuracy obtained is about 0.5 per cent. The main uncertainty, however, is in the absorption within the sample itself, which may in large samples be of the order of 10 per cent. When an estimate of the absorption is questionable the sample should be run by the radon method.

Another circuit sometimes used for rapid quantitative work, using a Geiger-Müller counter, is the counting rate meter. The circuit rectifies the pulses and applies them to a tank circuit with a time constant of the order of seconds in such a way as to produce a voltage across it proportional to the average frequency of pulses; this voltage is applied to an electronic voltmeter giving a direct meter reading proportional to the intensity of the radiation.

(3) *The radon method of analysis* determines the amount of radon collected in a sample over a known period of time from which the amount of radium is determined, independent of the presence of other radioactive substances and independent of absorption of the sample. The method using an electroscope is described in *Methods of Analysis, A.O.A.C.*, 552 (1940). This particular apparatus, using a Wulf electroscope, was able to measure quantities as small as 5×10^{-11} g. If a high sensitivity string electrometer is used in a constant temperature box and complicated optical system to record the trace of the image of the fiber on photographic record paper, the smallest amount detected is of the order of 10^{-14} grams of radium. A simpler, more rugged method for determining these small measurements is the alpha particle counting method.[2] This method uses the same procedure for deemanating samples and standard solutions as in *Methods of Analysis, A.O.A.C.*, 552 (1940), except that it is necessary to remove oxygen, carbon dioxide, and any other impurities in the gas which have an affinity for electrons. The oxygen is removed by reduced copper in a furnace and the carbon dioxide by ascarite, which also removes any acid vapors. A cylindrical chamber is used with an insulated central electrode. The chamber is maintained at a high negative potential of -500 to -1000 volts and the central rod is connected directly to the grid of the first stage of amplification. For each alpha particle emitted, a cloud of ions and electrons is formed; the electrons possessing high mobility are swept rapidly to the central electrode, thus producing an

[1] H. Franz and C. Weiss, *Physik ZS*, 36, 486 (1935).
[2] Leon F. Curtiss and Francis J. Davis, *Nat. Bur. Standards, U. S., J. Res.*, 31, Sept. 1943.

electronic pulse. This pulse is amplified from 10,000 to 20,000 times through an inverse feedback amplifier. A scaler is used to scale the frequency of pulses down by a constant factor so that a printing traffic recorder is able to count them. The error in either the electrometer or alpha particle counting method is determined essentially by the statistical variation of the total count of alpha particles. The lower the background, the greater the accuracy. Consequently, when only one chamber is used, as in the counting method, a lower background may be expected than when two chambers are used, as in the electrometer method.

The upper limit of actual sample placed in condenser for radon collection for the alpha particle counting method is in the neighborhood of 10^{-8} gm. of radium. Samples larger than this should be measured by taking an aliquot part. The accuracy of the analysis depends on the size of the sample and for very small samples the statistical variation of the count of the alpha particles is the predominant factor; for large samples the reproducibility is of the order of 1 per cent.

Any radon method of analysis requires considerable experience for operation. It should be run continuously with frequent checks of background and standard samples. It should require at least one experienced person's full time for operation to maintain equipment in operating condition. The alpha particle counting method requires an operator with an understanding of electronic circuits, such as high gain amplifiers, trigger circuits, etc. In view of the fact, as stated, that such equipment actually requires continuous operation to secure reliable results, in those situations where samples are measured at intervals of several weeks the cost is prohibitive. Much better results, at lower cost, can be achieved by sending such samples to a laboratory maintaining the required apparatus.

RECOMMENDATIONS*

It is recommended—

(1) That measurements by the electroscope method be continued, in particular for large samples of radium.

(2) That measurements by Geiger counter method be continued.

(3) That samples requiring the radon method of analysis, especially those containing less than 10^{-10} gms. of radium, be analyzed by a laboratory maintaing an alpha particle counting method, such as the National Bureau of Standards.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 46 (1945).

REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

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

Nine topics were assigned to associate referees for this year. The topic "Theophylline Sodium Salicylate" was later dropped because the Associate Referee cound find no such product appearing in commerce. Because of the scarcity of quinine during the War, it was also decided to drop the topic "Quinine and Strychnine" temporarily, until these products again appeared on the market. Of the remaining seven topics, reports were received on two, no reports on three, and short progress reports on two.

RECOMMENDATIONS*

It is recommended that a topic be set up on Quinine to study the titration method described by Herd,[1] and that a topic be set up on Ephedrine to restudy present assay methods for this substance.

It is further recommended that the present tentative method for alkaloids in ergot be deleted from *Methods of Analysis, A.O.A.C.* (1945), since it does not measure the total alkaloids.

Separate recommendations on editorial changes for the sixth edition of *Methods of Analysis, A.O.A.C.* (1945) have been made to the proper committee.

Chemical Methods for Ergot Alkaloids.—Because of pressure of wartime activities, it was found impossible to make satisfactory arrangements for a collaborative study on the chemical assay of ergot during the past year. Arrangements are now being made to have this work done. It is recommended that the topic be continued.

Physostigmine in Ointments.—No report received. It is recommended that the topic be continued.

Quinine Ethyl Carbonate.—No report received. It is recommended that the topic be continued.

Theobromine and Phenobarbital.—Several methods for the separation and determination of theobromine and phenobarbital in admixture have been investigated during the past year. As yet, none of these methods have proved to be entirely satisfactory. It is recommended that the topic be continued.

Prostigmine.—The Associate Referee recommends† that the present method be adopted as tentative; the Referee concurs, but recommends that a further collaborative study be made on an authentic mixture prepared by the Associate Referee for the purpose of determining the accuracy of the method. The results previously submitted in the report of

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 47 (1945).
[1] *J. Am. Pharm. Assoc.*, 31, 9 (1942).
† Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

the Associate Referee[2] were obtained on a commercial tablet, and while they agreed closely among the collaborators, the theoretical amount of prostigmine was unknown.

Aminopyrine, Ephedrine, and Phenobarbital.—The Associate Referee reports fair agreement among collaborators on the aminopyrine and phenobarbital but poor agreement on the ephedrine results. He recommends that the subject be continued and the Referee concurs.

Polarograph Methods.—No report received. It is recommended that the topic be dropped.

No reports were given by the Associate Referees on the following subjects: Chemical methods for ergot alkaloids, theophylline sodium salicylate, physostigmine in ointments, quinine ethyl carbonate, and theobromine and phenobarbital.

REPORT ON PROSTIGMINE

By F. J. McNALL (Food & Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

The Volhard procedure for the estimation of prostigmine bromide in tablet mixtures was studied last year and reported (*This Journal*, **26**,

TABLE 1.—*Report of collaborators*

COLLABORATOR	PROSTIGMINE BROMIDE
	per cent
1	7.05
	7.13
2	7.25
	7.22
3	7.40*
	7.36*
4	7.07
	7.07
5	7.12
	6.99
Associate Referee	7.00
	7.03
Average	7.09

* Blank not determined on reagents, therefore results not included in average.

[2] F. J. McNall, *This Journal*, **26**, 310 (1943).

310). In accordance with the recommendations of Subcommittee B, the work on prostigmine bromide was continued with special reference to a method which would be specific for prostigmine.

Prostigmine bromide or methylsulfate can be hydrolized with hot alkali with the formation of dimethylamine which can be subsequently distilled and collected in a measured amount of standard acid. This type of assay was submitted for collaborative study this year.

Tablets of prostigmine bromide were secured from a reputable manufacturer and prepared by grinding in a mortar to pass through a 60-mesh screen. The proposed method was submitted to 5 collaborators.

The Associate Referee wishes to express his appreciation to the following collaborators, all members of the U. S. Food and Drug Administration: Rupert Hyatt, Cincinnati, Ohio, M. J. Meszaros, Chicago, Ill., Ruth R. Segall, Chicago, Ill., and H. W. Conroy and H. P. Bennett, Kansas City, Mo.

DISCUSSION

The method as outlined is one that is more or less specific for prostigmine salts. No adverse criticism was received from any of the collaborators and the results are in good agreement.

RECOMMENDATIONS*

It is recommended that the method as outlined be made tentative for tablets of prostigmine bromide.

REPORT ON AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL

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

The determination of these drugs in admixture was assigned for investigation in 1941, attention being called to certain correspondence indicating difficulty in obtaining concordant results on determinations of ephedrine by A.O.A.C. and N.F. methods. This was largely due to losses by volatilization and it was corrected by the procedures adopted in later revisions which eliminate the evaporation of the ether solution of the base.

At that time, the Associate Referee was able to discover only one commercial preparation containing these three drugs, and no others have come to his attention since.

The separation of aminopyrine from ephedrine (or other alkaloids) presents some difficulty on account of the basic properties of aminopyrine, but it was soon found that from a solution of *p*H about 4.5, the amino-

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 47, 85 (1945). Details of the method will appear in *Methods of Analysis, A.O.A.C.*, 6th ed., 1945.

pyrine could be completely extracted by ether without removing any ephedrine, at least when tartaric acid was used. It happens that the use of sodium bitartrate solution gives the acidity required. In the method tried, phenobarbital is extracted with the aminopyrine, and these are then separated by the use of dilute sulfuric acid, which holds the aminopyrine, allowing the phenobarbital to be extracted with chloroform. The aminopyrine is finally removed by adding excess of ammonia and chloroform extraction. The ephedrine is extracted from the bitartrate solution after addition of excess sodium hydroxide, using essentially the N.F. procedure.

The method of separation outlined above was submitted in more detail to four collaborators, with a mixture approximating the proportions of these drugs in the commercial product, together with lactose, starch, and talc.

Results reported were quite satisfactory for both phenobarbital and aminopyrine, but showed considerable variation in the case of ephedrine, the reason for which is not apparent.

I recommend* that this and other methods be studied further with a view for improving the accuracy of the ephedrine determination.

No report was given on quinine and strychnine nor on polarographic methods.

REPORT ON SYNTHETIC DRUGS

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

Nineteen topics were considered this year and two contributed papers were received. One topic was not studied because no Associate Referee could be found who was willing to undertake it. Analytical procedures for six drugs were considered by the Associate Referees and the Referee to have sufficient merit to warrant recommendations for adoption as tentative methods. Progress was reported by the Associate Referees in five other subjects. One Associate Referee reported that no work had been done on his subject and asked to be relieved of his assignment. No reports were received from nine other Associate Referees. One Associate Referee recommended that one method be adopted as tentative in his field but asked that the topic be continued, in order that more collaborative work might be done.

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* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 47 (1945). The details of the method will be published in *Methods of Analysis, A.O.A.C.*, 6th ed., 1945.

RECOMMENDATIONS*

The Referee recommends that the following new subjects be studied: Effervescent antipyrine with caffeine; dihydrocodeinone; butacaine sulfate; spirit of camphor.†

It is recommended that the status of the following methods be advanced from official, first action, to official, final action:

Acetophenetidin and caffeine (*Methods of Analysis*, **XXXIX**, 16, 17).
Bismuth compounds in tablets (*Ibid.*, 178).
Calcium gluconate (*Ibid.*, 179).
Effervescent potassium bromide with caffeine (*Ibid.*, 202, 203).
Iodine (*Ibid.*, 183).
Mandelic acid (*Ibid.*, 154).
Oil of chenopodium (*Ibid.*, 208).
Phenolphthalein in chocolate preparations (*Ibid.*, 162).
Sulfanilamide (*Ibid.*, 168).
Theophylline (*Ibid.*, 107).

The Associate Referee recommends that the status of the microchemical methods for the alkaloids or synthetics listed below be advanced from tentative to official, final action under suspension of the rules.

Berberine, *Methods of Analysis*, **XXXIX**, 222; and *This Journal*, **22**, 88, 706.
Cotarnine, *Ibid.*, 222; and *This Journal*, **22**, 88, 706.
Narceine, *Ibid.*, 222; and *This Journal*, **22**, 88, 706.
Narcotine, *Ibid.*, 222; and *This Journal*, **22**, 88, 706.
Physostigmine, *Ibid.*, 222; and *This Journal*, **23**, 55, 746; **24**, 92.
Stovaine, *Ibid.*, 222; and *This Journal*, **23**, 55, 746.
Benzedrine, *This Journal*, **25**, 105 (1942).
Choline, *Ibid.*, **27**, 110 (1944).
Dilaudid, *Ibid.*, **24**, 92 (1941).
Metrazol, *Ibid.*, **25**, 106 (1942).
Sodium sulfapyridine, monohydrate, *Ibid.*, **24**, 92 (1941).
Sulfadiazine, *Ibid.*, **27**, 110 (1944).
Sulfapyridine, *Ibid.*, **24**, 93 (1941).
Sulfathiazole, *Ibid.*, **25**, 106 (1942).

The Referee concurs.

The Referee recommends that the status of the following named methods be advanced from tentative to official, first action:

Acetophenetidin, acetylsalicylic acid, and salol, *Methods of Analysis*, **XXXIX**, 34, 35; *This Journal*, **23**, 89, 752 (1940).

Acetylsalicylic acid and phenophthalein in tablets (*Ibid.*, 36, 37; *This Journal*, **21**, 560 (1938); **22**, 95, 732 (1939)).

Cinchophen, *Ibid.*, 142, 143; *This Journal*, **20**, 83, 589 (1937); **21**, 95, 554 (1938).

Cod liver oil in emulsions, *Ibid.*, 207; *This Journal*, **22**, 96, 739 (1939).

Mercurous iodide in tablets, *Ibid.*, 193; *This Journal*, **12**, 52 (1939).

Mercury in ointment of mercuric nitrate, *Ibid.*, 197; *This Journal*, **22**, 96, 743 (1939).

* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 48, 84 (1945).

† Results of an experimental procedure for modification of the U.S.P. Assay for Spirit of Camphor, by Harry W. Conroy, are published in *This Journal*, p. 719.

Methenamine in tablets, *Ibid.*, 53; *This Journal*, **3**, 374, (1920).
Nicotinic acid in tablets and ampuls, *Ibid.*, 156; *This Journal*, **23**, 89, 765 (1940).
Nitrites in tablets, *Ibid.*, 199; *This Journal*, **18**, 89, 544 (1935).
Pyridium, *Ibid.*, 165; *This Journal*, **20**, 576 (1937); **21**, 94, 552 (1938).
Sampling, *Ibid.*, 1; *This Journal*, **10**, 99 (1927).
Sulfonal and trional, *Ibid.*, 66; *This Journal*, **16**, 83, 366 (1933).
Theobromine in theobromine calcium, *Ibid.*, 104; *This Journal*, **19**, 105 (1936).
Volatile acidity of tragacanth, *Ibid.*, 127; *J. Ind. Eng. Chem.*, **4**, 374 (1912); *Bur. Chem. Circ.*, 94, p. 1.
Aminopyrine, acetophenetidin, and caffeine, *This Journal*, **24**, 91, 809 (1941).

The Referee recommends that the tentative method for chloroform in mixtures be deleted (*Ibid.*, 130).

Chloroform and Carbon Tetrachloride.—The former Associate Referee recommended that the status of the tentative method for chloroform and carbon tetrachloride (*Ibid.*, 132) be advanced to official, final action under suspension of the rules. The Referee concurs.

Ipomea and Jalap.—It is recommended that the tenative method for ipomea and jalap be reinstated (*This Journal*, **16**, 84, 1933).

Terpin Hydrate in Elixirs and Terpin Hydrate and Codeine in Elixirs.—Three associate referees have worked on different phases of this topic (*This Journal*, **11**, 358, 1928; *Ibid.*, **15**, 415, 1932; *Ibid.*, **23**, 757, 1940). Comments by various drug analysts have indicated that of the two tentative methods for determining Terpin Hydrate in Elixirs (**XXXIX**, 63, 64, and 65**(a)**, the one in 65 **(a)** is superior. The two latter Associate Referees recommend that the tentative method for Terpin Hydrate in Elixirs (**XXXIX**, 63, 64) be deleted. The Referee concurs.

The Referee further recommends that the tentative method for Terpin Hydrate and Codeine (**XXXIX**, 65 (a) and (b)) be advanced to official, first action.

Cinchona Alkaloids.—Several complaints have been received that the A.O.A.C. Method (1940) for the separation of the four principal alkaloids of cinchona is unreliable (**XXXIX**, 69, 70). The Referee has consulted with several chemists experienced in the analysis of cinchona preparations and is informed that in their opinion the method is not accurate. The Pharmacopoeia has adapted methods for the determination of these alkaloids in Totaquina. Several chemists consulted consider these methods superior to the A.O.A.C. procedure. It is recommended that the A.O.A.C. method be deleted.

Benzedrine in Inhalants.—Three years ago (*This Journal*, **25**, 104, 1942) a benzoylation method for the determination of benzedrine was adopted tentatively by the Association. The subject was continued in order to study methods for the determination of benzedrine in inhalants and other pharmaceuticals. Since that time no formal report has been submitted. The Associate Referee has requested that he be relieved of further study on this topic since he states that he is more interested in

other subjects. The Referee considers this an unimportant topic and recommends that it be dropped.

Phenothiazine.—Three years ago the Association decided to study phenothiazine, but no report was received last year before the meeting and the topic was continued. In the meantime the N.F. VII included phenothiazine and provided a colorimetric assay.

This year two reports were received and the first one published (*This Journal*, **27**, 343).[1] The Associate Referee has done considerable work on the topic and has devised a method which he considers superior to the N.F. procedure. The method is an adaptation and combination of published procedures, using bromine water as reagent and photoelectric colorimeter. This method has been applied, by the Associate Referee and his collaborators, to commercial phenothiazine, to mixtures of the drug with starch, with lactose, with talc, and also to commercial tablets. The results are as good as may be expected from this type of method.

The Associate Referee recommends that the method devised be adopted as a tentative method, but that the topic be continued in order to secure more data by collaborative study. The Referee concurs.

Hydroxyquinoline Sulfate.—This topic has been assigned for several years but until this year no formal reports of progress had been submitted. The Associate Referee was unable personally to do any work on the topic but was able to direct some studies by an assistant. A method of assay by titration with bromide-bromate solution was developed which appears promising. It is recommended that the subject be continued.

Methylene Blue.—This year the studies were confined to an investigation of the Deahl and Maurina method (*J. Am. Pharm. Assoc.*, **32**, 301, 1943). The results obtained on the pure substance by this method were good, but the analyses of tablets were not satisfactory.

The Associate Referee recommends that the subject be continued. The Referee concurs.

Ethylaminobenzoate (*Benzocaine*).—The Associate Referee investigated three methods for the assay of benzocaine. The bromometric procedure was found the most satisfactory. In collaborative trials this gave results about 0.3 per cent too high. The Associate Referee recommends that the method be adopted as a tentative method. The Referee concurs.

Benzocaine Ointment.—A method for the extraction of benzocaine from its ointment was developed, after which the bromometric method was applied. The results obtained in collaborative trials were good.

The Associate Referee recommends that the method developed by him and his collaborators be adopted as tentative. The Referee concurs and recommends that the topic be closed.

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

[1] The second is printed in *This Journal*, p. 693.

Barbiturates.—The Referee recommends that the present method for barbital and phenobarbital be dropped and that Method No. II in his report be substituted therefor under the general heading "Barbiturates." He further recommended that the method be adopted as official, final action under suspension of the rules, and that the subject be closed.

Phenolphthalein in the Presence of Bile Salts.—Last year the Associate Referee developed a method for the determination of phenolphthalein in bile-salt mixtures which gave good results, but no collaborative work was done (*This Journal*, **27**, 353, 1944). It is recommended that the topic be continued.

Atabrine (*Quinacrine Hydrochloride*).—This substance was assigned three years ago, but no meeting was held in 1942 and no report was submitted. In the meantime, the drug and tablets of it had been admitted to the Pharmacopoeia and an assay provided for each. Since some work has been done by the Associate Referee, a method of assay devised, and a report submitted last year (*This Journal*, **27**, 354, 1944), it was decided to continue the study this year. No report was received. It is recommended that the subject be continued.

Sedormid.—Last year the Associate Referee devised a method by which sedormid is decomposed in acid solution (HCl 1+1) to form urea. By the action of urease the urea is converted into ammonia. The method gave good results on the pure chemical but they were unsatisfactory on tablets. Later the acidity was increased (HCl 3+2) and the results were better. This year the urease method was abandoned. By extracting the tablets with chloroform the sedormid was obtained in a sufficiently pure state to be weighed. Recoveries of 99–100 per cent were obtained. The Associate Referee recommended that the method be adopted as tentative. The Referee concurs. The Referee recommends that the topic be closed.

Demerol (*Isonipecaine*).—Demerol is a synthetic which is used as a substitute for morphine. It was recently classed as a narcotic by Congress under the name of "isonipecaine." The Associate Referee developed two methods for the assay of demerol. One depends on distillation in the presence of a mild alkali (calcium carbonate) followed by titration of the distillate. The other is an extraction procedure. The results by both methods on the pure substance and on mixtures simulating tablets were good. The Associate Referee recommends that the subject be continued. The Referee concurs.

Propadrine Hydrochloride.—This topic was assigned last year. No report was received. It is recommended that the subject be continued.

Carbromal.—Carbromal was introduced as "Adalin." It was official in the U.S.P. XI and is now described in N.F. VII. No method of assay is described in either compendium. The Associate Referee applied an extraction method with solvents to tablet material, after which the drug was weighed. The identity and purity of the extracted material was deter-

mined by melting point and bromine content. The results were promising, but no collaborative work was done.

The Associate Referee recommends that the topic be continued. The Referee concurs.

Phenolsulfonephthalein.—This substance (also known as phenol red) is used as an indicator and to evaluate kidney function. A method of assay for the dye in solution is given in the U.S.P. XII. Several methods of assay, including the U.S.P., were tried on commercial samples of the dye. The results were not encouraging. This led to the conclusion that the material was not pure. No satisfactory method of purification was found. The Associate Referee recommended that the subject be dropped. The Referee concurs.

Procaine.—It is recommended that Method III for Procaine be adopted as official, first action (**XXXIX, 99**).

Methods for the assay of procaine have been adopted by the Association from time to time. One of these (Method I) was criticized on the ground that the time allowed for the bromination (30 minutes) was insufficient. At the request of the Referee this was investigated by an Associate Referee. The report indicates that the time prescribed by the A.O.A.C. method is sufficient. Therefore, no change in the method as published is recommended.

Sulfobromophthalein.—This substance is described in the U.S.P. XII, but no assay is provided. A method for the determination of bromine in organic compounds was developed by the Associate Referee and applied to sulfobromophthalein. The results were satisfactory, but no collaborative work was done. The Associate Referee recommends that the subject be dropped. The Referee concurs.

No report was given on benzedrine in inhalants by the Associate Referee; see report of Referee.

REPORT ON PHENOTHIAZINE

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

Preliminary studies conducted by the Associate Referee[1] indicate that of the few methods which have been suggested for the determination of phenothiazine the most satisfactory procedure is the electrophotometric one using bromine water as the reagent. Samples were prepared for a collaborative study of this method. A majority of the collaborators were un-

[1] *This Journal*, **27**, 343 (1944).

TABLE 1.—*Collaborative results*

SUBSTANCE	PHENOTHIAZINE—PER CENT										
	THEORETICAL	ANALYST NO.1[1]			ANALYST NO. 2[1]	ANALYST NO. 3				AVERAGE[2]	AVERAGE[3] DEVIATION FROM AVERAGE
		1	2	AVERAGE	AVERAGE	1	2	3	AVERAGE		
Commercial phenothiazine powder	?	96.0	95.1	95.5	100	98.8	98.8	98.8	98.8	98.1	±1.7
Lactose and phenothiazine	78.5	77.2	76.4	76.8	80	76.6	79.8	79.3	78.6	78.5	±1.1
Starch and phenothiazine	88.3	84.7	84.2	84.5	90.9	86.6	88.4	86.0	87.0	87.5	±2.3
Commercial tablets Phenothiazine Mfg. A	?	86.9	88.0	87.4	86.9	88.0	87.1	87.3	87.5	87.3	±0.2
Commercial tablets Phenothiazine Mfg. B	?	72.3	71.3	71.8	74.0	75.6	74.8	75.0	75.1	73.6	±1.2
Phenothiazine powder Medicinal Grade	?	98.0	98.2	98.1	100	100.7	99.2	99.4	99.8	99.3	±0.8

[1] Determinations made with filter.
[2] Average of the averages of the three analysts.
[3] Average deviation of the averages of the three analysts from the combined average

able to complete the analyses. Collaborators who have reported, in addition to the Associate Referee, are: M. I. Smith and W. T. McClosky, U. S. Public Health Service, National Institute of Health, Division of Physiology, Bethesda, Md., and I. S. Shupe, Winthrop Chemical Co., Inc., Rensselaer, N. Y. The Associate Referee deeply appreciates the assistance of these collaborators.

Samples submitted for analysis consisted of phenothiazine powder from two different manufacturers; phenothiazine tablets containing no other drugs, also from two different manufacturers; a mixture of lactose (20 per cent) and commercial phenothiazine powder (80 per cent); and a mixture of starch (10 per cent) and commercial phenothiazine powder (90 per cent). Theoretical phenothiazine content of the last two mixtures mentioned was calculated from the average phenothiazine content (as determined by the three analysts) of the commercial powder used in preparing the mixtures.

Smith and McClosky conducted a spectrophotometric analysis of the color produced by treating phenothiazine with bromine water. They report that it has a definite absorption band with a minimum of transmission in the region of 500–530 millimicrons. Accordingly they prepared electrophotometer calibration curves from data obtained without the use of a filter and also using a 525 millimicron filter. This study clearly indicates that more consistent results can be obtained by using a filter. The analyses of these collaborators were made with a Fisher electrophotometer using 5 ml. cells with an outside diameter of 13 mm.*

Shupe also made a spectrophotometric analysis of the colored solution and found a minimum of transmission in the region of 510 millimicrons. This wave length was used in his determinations. His analyses were made with a Beckman spectrophotometer, 0.04 mm. slit width, in 10 mm. cells.

Determinations made by the Associate Referee were conducted with a Fisher electrophotometer in 25 ml. cells with a depth of 10 mm. These determinations were made without the use of a filter. The instruments of all collaborators were calibrated by means of phenothiazine that had been carefully purified as described below.

The procedure followed by the collaborators, including modifications which have been suggested as a result of this study, will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

Table 1 reports the results of the analyses conducted by collaborators. These indicate that the method is not capable of extreme accuracy. The average deviation from the average of the results of all analysts for a given sample varies from $\pm 0.4\%$ to $\pm 2.3\%$. This is greatly in excess of the $\pm 0.2\%$ average deviation from the average observed by the Associate Referee in the previous report,[1] representing the work of only one analyst.

* The Collaborative report of McClosky and Smith follows this report (p. 696).
[1] *Loc. cit.*

The use of different makes of instruments may account for part of this variation. Other factors that may introduce errors have been previously discussed.

The method may be considered satisfactory for most purposes where extreme accuracy is not required. Analysts should bear in mind the limitations of the method in reporting results. The method has been shown to be applicable to phenothiazine powder and phenothiazine tablets containing only excipients. Preliminary studies indicate that the method is applicable also to phenothiazine tablets containing other drugs. Final results of these studies will be reported later.

RECOMMENDATIONS†

It is recommended—

(1) That the method presented be adopted as a tentative procedure.

(2) That collaborative study of the subject be continued in order to provide more data.

COLLABORATIVE REPORT ON EXPERIMENTS ON ASSAY OF PHENOTHIAZINE

By WILLIAM T. MCCLOSKY and M. I. SMITH (Division of Physiology, National Institute of Health, U. S. Public Health Service, Bethesda, Maryland)

Seven samples were received from the A.O.A.C. Associate Referee, Vincent E. Stewart, on phenothiazine, to be analyzed by a modification of the procedure given in *The National Formulary*, 7th Ed., page 323.

Before analysis it was necessary to calibrate the Fisher electrophotometer with a sample of pure phenothiazine. It was recommended by the Associate Referee that this be done without a filter, but during the course of the experiments it was found that the use of Fisher filter B 525 gave better results. It gives the method greater sensitivity and yields a higher degree of accuracy than could be obtained without a filter. The accompanying graph of a spectrophotometric analysis of a pure sample of phenothiazine, oxidized with bromine according to standard procedure, shows clearly that there is a definite absorption band, with a minimum of transmission in the region of 500–530 mμ. It would therefore follow that a filter of this type should give best results. This is fully confirmed by the analytical data in Figure 2 in which Curve A gives the results with the B 525 filter and Curve B, the results with no filter. The series of standard drug concentrations was the same in both cases. The results on the standards are shown in greater detail in Table 1; the results with the unknowns (using the same analytical procedure) are shown in Table 2. It would

† For report of Subcommittee B and action by the Association, see *This Journal*, 28, 48 (1945). Details of the method will be published in the 6th edition, *Methods of Analysis*, 1945.

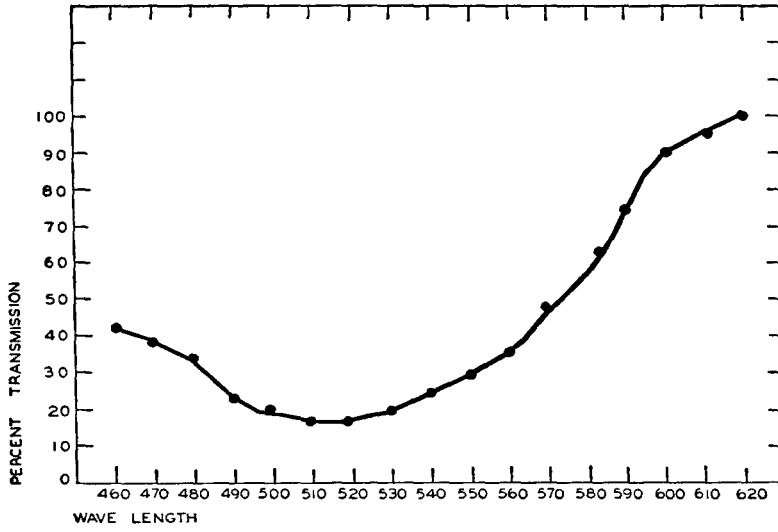


FIG. 1.—Spectrophotometric analysis of pure phenothiazine.

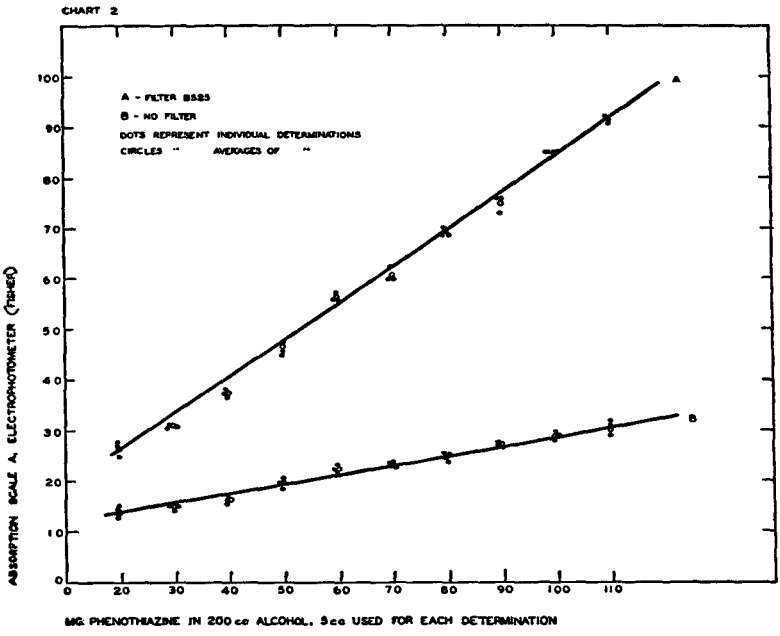


FIG. 2.—Results of analysis with and without filter.

TABLE 1.—*Electrophotometer readings, absorption scale A*
(5 ml. used for oxidation with bromine in all cases)

PHENOTHIAZINE	NO FILTER		FILTER B 525	
	INDIVIDUAL READINGS	AVERAGE	INDIVIDUAL READINGS	AVERAGE
mg/200 ml. alcohol				
20	14.3		27.0	
	14.0		27.1	
	13.0	13.8	25.0	26.4
30	14.5		31.0	
	15.0		31.0	
	14.5	14.7	31.0	31.0
40	16.0		38.0	
	16.1		37.0	
	16.5	16.2	37.5	37.5
50	20.5		47.5	
	18.5		45.0	
	19.5	19.5	46.0	46.2
60	23.0		57.0	
	21.5		56.0	
	22.3	22.3	56.0	56.3
70	23.5		60.0	
	23.0		60.0	
	23.5	23.3	62.5	60.8
80	25.0		70.0	
	25.0		69.0	
	24.0	24.7	69.0	69.3
90	27.0		76.0	
	27.2		76.0	
	27.0	27.1	73.0	75.0
100	29.5		85.0	
	29.3		85.0	
	28.5	29.1	85.0	85.0
110	31.0		92.0	
	30.7		92.0	
	29.0	30.2	91.0	91.7

appear that the potency of the unknowns varies from 74 to 100 per cent of the standard. The microcells used had an outside diameter of 13 mm. and a capacity of 5 ml. All readings were made on the A (absorption) scale of the electrophotometer.

TABLE 2.—*Assay of unknowns.*

(In all cases 5 ml. used for oxidation with bromine)

SAMPLE NUMBER	MG./200 ML. ALCOHOL	NO FILTER		FILTER B 525		% OF STANDARD
		INDIVIDUAL READINGS	AVERAGE	INDIVIDUAL READINGS	AVERAGE	
2	100	30.2 28.0	29.1	85.0 85.0	85.0	100
3	125	30.5 27.5	29.0	85.0 85.0	85.0	80
4	110	27.2 28.6	27.9	83.0 85.0	84.0	90.9
5	115	29.9 28.6	29.3	86.0 86.0	86.0	86.9
6	135	28.0 27.5	27.8	85.0 85.0	85.0	74.0
7	100	27.1 28.1	27.6	85.0 85.0	85.0	100

No report on plasmochine was given by the Associate Referee.

REPORT ON 8-HYDROXYQUINOLINE SULFATE

By E. H. GRANT,* and A. M. ALLISON† (Food and Drug Administration, Federal Security Agency, Boston, Mass.)

Work this year was confined to the determination of this chemical by bromination methods. According to Beilstein (1) (referring to various authors), the action of bromine on 8-hydroxyquinoline yields 5-brom-8-hydroxyquinoline, 5-7-dibrom-8-hydroxyquinoline, and finally 3-5-7-tribrom-8-hydroxyquinoline, also several additional products of unspecified composition. Of these reactions, the bromination to 5-7-dibrom-8-hydroxyquinoline can be most easily controlled and measured.

This 5-7-dibrom-8-hydroxyquinoline is a white solid, readily crystallizing, m.p. 196° uncorrected (1). It is sufficiently soluble in chloroform that it may be extracted therewith and sufficiently nonvolatile that it may be dried for short periods at 100°C. and weighed.

E. Schulek and O. Clauder (2) have published a method for the bromotometric determination of hydroxyquinoline by adding an excess of

* This study conducted by E. H. Grant.
† Associate Referee in 1944.

standard bromide-bromate solution, then hydrochloric acid, stoppering, allowing to stand 5 min., adding potassium iodide and titrating the excess iodine. The then Associate Referee tried this method once in 1941, obtained a result of 101.8 per cent of theory, and abandoned the method, without reporting his findings.

The present author has attempted to develop a similar method, either determining the excess bromine or destroying it and extracting and weighing the dibromhydroxyquinoline. A study was made of the effects of changes in various variables. The results indicated that as long as appreciable amounts of free bromine are present, bromination cannot be stopped accurately at the desired point. In this respect, hydroxyquinoline acts very much like phenolsulfonates (3).

Experiments were then performed titrating the hydrochloric acid solution of hydroxyquinoline with 0.1 *N* bromide-bromate solution, with the use of dye as indicator, in a manner similar to the present official method for phenolsulfonates (4). Methyl orange is too easily brominated to be used in the present instance, but satisfactory results were obtained with methyl red. The rates of the various reactions involved are affected greatly by the temperature of the medium. If ice be added, no end point can be ascertained. At room temperature, the titration can be conducted, but the end point is quite unsatisfactory. This is very much better at 50° or 70°C. Results using 0.1 *N* Br had a tendency to run about 0.1 per cent higher at 50°C. than at room temperature. The supply of hydroxyquinoline used was not pure enough to permit definite conclusion as to which result is the most correct.

Hydroxyquinoline sulfate is used as an antiseptic in several preparations, often in quite high dilutions. It is therefore desirable to develop a method applicable to very small amounts of this chemical. For this reason, a study was made of titration with 0.01 *N* Br. At this dilution, quite low results were obtained at 50°C., but satisfactory ones at 70°. At this higher temperature, the bleaching of the methyl red is very rapid and excessive amounts of indicator are required. To overcome this, most of the titration was performed at 50° and it was completed at 70°.

Hydroxyquinoline is very weakly basic. If chloroform is shaken with an acid solution of hydroxyquinoline, the partition of this between the two solvents will depend on the *p*H of the aqueous layer. If the slightly acid aqueous solution is buffered with the salt of a weak acid, the hydroxyquinoline may be extracted with chloroform, and then extracted from the chloroform by using hydrochloric acid of sufficiently high concentration. Because of the phenolic nature of the hydroxyl group, hydroxyquinoline is not easily extracted from sodium hydroxide solution, but can be extracted from a sodium bicarbonate or borax solution. Free hydroxyquinoline is quite volatile; indeed, steam distillation has been suggested as a means of

isolating this base. It is not volatile from a 4 per cent hydrochloric acid solution and boiling such a solution does not lower its titration.

Two methods are given below for the determination of hydroxyquinoline. The first is the simplest and most accurate and is to be used whenever enough of that chemical is present in a sufficiently small volume. The second is intended only for the determination of minute amounts.

METHODS

REAGENTS

(a) *Bromide-bromate solution.*—0.1 *N* Br. [See *Methods of Analysis*, **XXXIX, 26 (c)**].

(b) *Bromide-bromate solution.*—0.01 *N* Br. Dilute one volume of (a) accurately to ten volumes.

(c) *Methyl red solution.*—See *Methods of Analysis*, **XXXIX, 82 (b)**.

(d) *Diluted methyl red solution.*—Dilute 1 volume of (c) to 4 volumes with water and sufficient sodium hydroxide to dissolve the dye.

DETERMINATION. METHOD I

(For amounts of hydroxyquinoline sulfate between 25 and 250 mg.)

According to the nature of the other ingredients in the sample, extract either from an acid or an alkaline solution. If an acid medium is chosen, adjust with ammonia to very slightly acid and add 1 gm. ammonium acetate per 100 ml. of solution.

If an alkaline medium is chosen, either sodium bicarbonate or borax, but not sodium hydroxide, may be used. Ammonium salts are undesirable in an alkaline shake out.

In either case, extract the adjusted solution with several portions of chloroform; usually 6, totalling 70 ml., are sufficient. A test for complete extraction may be made by adding a little 4% HCl to last portion, evaporating the chloroform on steam bath, adjusting to about 70°, adding a drop of 0.01 *N* Br and then a drop of diluted methyl red, which should be bleached immediately. Extract combined chloroform extracts with 5 portions of a mixture of 1 volume HCl and 9 volumes H_2O, combining all portions. If interfering substances are probably present, wash acid extracts with small fresh portion of chloroform and wash this with fresh portion of acid, adding this to the other acid extracts. If the sample contained phenol or other volatile, undesirable substances, and these have not been completely removed by preceding process, boil acid soln to remove them, maintaining volume approximately constant by adding more water. (If sample is of such purity that extraction is not necessary, dilute sample to 75 ml. and add 5 ml. HCl.)

Adjust acid soln to 50°C. and maintain it at this temperature during the titration by occasionally reheating. Add a drop (or more, if needed) of the methyl red soln (c) from a buret and titrate with the 0.1 *N* Br. As this is added, the color of the liquid gradually changes from brown orange to yellow. Add more indicator whenever the yellow is about reached. At slightly beyond half-way point in titration, dibromhydroxyquinoline will start to crystallize out, if the concentration is great enough. Sometimes the crystals absorb some dye and persistently retain a slight pink or orange color. Disregard color of the precipitate; judge by that of the soln. (By diluting to not over 0.1 gm. hydroxyquinoline sulfate per 100 ml., the formation of precipitate can be avoided.) The end point is reached when, after waiting 10 sec. for the absorption of the last drop of Br and adding a drop of indicator, it is bleached almost instantly. The timing for the addition of last drop of indicator at the end point is important, as the proper conditions prevail for but a brief period.

Read the volumes consumed of two solns. Measure 10 ml. of the methyl red soln

into an Erlenmeyer flask, add 2 ml. HCl and titrate with 0.1 *N* Br. Correct main titration for the amount of Br consumed by the volume of indicator used. 1 ml. 0.1 *N* = .0036265 gm. 8-hydroxyquinoline or .004853 gm. 8-hydroxyquinoline sulfate (anhydrous).

METHOD II

(For amounts between 2 and 10 mg.)

Extract as in Method I. Limit amount of acid mixture to 30 ml., divided into 5 portions, if possible. Titrate as in Method I, using 0.01 *N* Br and the diluted methyl red soln. Use least amount of indicator possible. No dibromhydroxyquinoline precipitates during titration. When the end point is almost reached, evidenced by a drop of indicator lasting only for about 0.5 ml. of the titrating soln, heat acid soln to 70°C. and complete titration at this temperature.

The correction for the indicator is usually negligible when using 0.1 *N*, but amounts to about 0.25 ml. for each 1 ml. of indicator used with 0.01 *N*.

Results by these methods are shown in Tables 1 and 2.

TABLE 1.—*Using 0.1 N Br*

EXPERIMENT NO.	SAMPLE USED	0.1 N Br, CORRECTED	FOUND
	8-HYDROXYQUINOLINE		
	gm.	*ml.*	*per cent*
1	0.16	43.7	99.05
2	.16	43.6	98.8
3	.16	43.6	98.8
4	.16	43.7	99.05
5	.16	43.7	99.05
6	.16	43.75	99.16
7	.16	43.85	99.39
8	.16	43.75	99.16
9	.16	43.75	99.16
	8-HYDROXYQUINOLINE SULFATE		
	gm.	*ml.*	*per cent*
10	0.24	44.95	90.89
11	.24	45.1	91.20
12	.24	45.05	91.09
13	.24	45.1	91.20
14	.25	46.87	90.97

Details of experiments. All titrations without previous extraction, unless otherwise noted:

1, 2, 3 titrated at 25°.

3 extracted from acid soln as in Method I.

5 started at 25°, finished at 70°C.

6 through 12 started at 25°, finished at 50°C.

4, 13, 14 exactly as Method I. 14 extracted from $NaHCO_3$ soln.

TABLE 2.—*Using 0.01 N Br*

8-HYDROXYQUINOLINE			
EXPERIMENT NO.	SAMPLE USED	0.01 *N* Br, CORRECTED	FOUND
	gm.	*ml.*	*per cent*
15	0.004	10.6	96.1
16	.004	10.9	98.8
17	.004	11.0	99.7
18	.004	10.97	99.5
19	.004	10.95	99.3
20	.004	10.83	98.2
21	.004	10.9	98.8
22	.004	10.8	97.9

Details of experiments. 15, titrated at 50°C. Others as Method II:

19. Extracted from 500 ml. of an almost saturated soln of commercial salt, acid medium. This represents a dilution of 1 in 125,000.
20. Extracted from 5 gm. $NaHCO_3$ in 500 ml. of H_2O.
21. Boiled 30 min. in acid solution.
22. Extracted from 250 ml. of saturated borax solution.

These methods were submitted to two analysts of this Station, who reported as follows:

TABLE 3.—*Results of analyses*

ANALYST	METHOD	NO. OF ANALYSES	WT. SAMPLE	FOUND (AVE.)
			gm.	*per cent*
Charles S. Purcell	IA	5	0.064	99.62
	IC	1	.064	99.50
	IIA	5	.0064	99.31
Frances C. Maguire	IA	4	.16	99.36
	IB	1	.16	98.00
	IIA	2	.004	99.09

A, without extraction; B, extracted from acid; C, from $NaHCO_3$.

All workers agree that a little experience is necessary to judge the end point, but that when this is acquired the method is quite workable. Hydroxyquinoline gives a yellow color both before and after bromination and the brominated methyl red is also yellow, fortunately all quite pure yellows. It is a little difficult to judge color changes against the yellow background. Experiments were run with Lovibond glasses, combining various quantities of yellows and reds, also with dyes of different colors and ease of bromination, and it was decided that methyl red is probably about the best dye indicator which can be hoped for. Another drawback is that, near the end point, if the indicator is added too soon, before the bromine has combined to form dibromhydroxyquinoline, the dye will be

bleached, even though the end point has not been reached. On the other hand, free bromine will not remain long in the solution, but soon attacks the dibromhydroxyquinoline to form higher brominated compounds. Therefore the analyst must not wait too long before adding the indicator.

The 8-hydroxyquinoline and its sulfate used in the above experiments were from Eastman Kodak Company, Nos. 794 and 1776 in their list. Both of these gave evidences of being not completely pure. The exploratory work was performed with the base, which was the purer of the two. Two other samples of hydroxyquinoline sulfate were on hand, assaying, by Method I, 91.76 per cent and 95.45 per cent, respectively. The last had much the best appearance of the three lots. As a result of his work with a phosphotungstic acid method, Reo E. Duggan (5) concluded that the commercial supplies of the sulfate are the monohydrate. This salt would contain theoretically 95.57 per cent of anhydrous hydroxyquinoline sulfate. On this basis, the three samples above are, respectively, 95.3 per cent, 96 per cent, and 99.87 per cent pure.

Hydroxyquinoline sulfate may be detected by adding a slight excess of bromine water to an aqueous solution of the sample, or to the hydrochloric acid solution of hydroxyquinoline extracted as suggested above, extracting the dibromhydroxyquinoline with chloroform, evaporating to a small volume, placing a drop on a microscope slide and examining the crystals. These are long, flat needles. If they form in the solvent at rest, they intersect in bundles; if the solvent is agitated, they are separate. If the solvent is evaporated rapidly before crystallization starts, they form a branching, fern-like pattern over the entire field. These crystals may be dissolved in alcohol and a solution of ferric chloride added. A green color is produced. By this method, 0.1 mg. of hydroxyquinoline sulfate dissolved in 500 ml. of water was easily detected.

The work next year should include the obtaining of some purer material on which to test the method, the examination of commercial preparations containing hydroxyquinoline sulfate, and the submission of samples to collaborators. It is recommended* that the subject be continued.

REFERENCES

(1) Beilstein's "Handbuch der Organische Chemie," 1942, **21**, 91 and 97.
(2) E. SCHULEK and O. CLAUDER, *Ber. ungar. pharm. Ges.*, **13**, 165–78 (1937) thru Chem. Abstracts, **31**, 3418 (1937).
(3) E. H. GRANT, *This Journal*, **14**, 351; **15**, 424; **16**, 364 (1931–2–3).
(4) *Methods of Analysis, A.O.A.C.*, 1940, 623, 634.
(5) *This Journal*, **25**, 796 (1942).

* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 48 (1945).

REPORT ON METHYLENE BLUE DETERMINATION IN MIXTURES

By HARRY O. MORAW (U. S. Food and Drug Laboratory, Chicago, Ill.), *Associate Referee*

No work was done on this subject in 1943. In 1942, a brief report published in *This Journal* (26, 242) concluded that by use of the A.O.A.C. separation procedure, methylene blue could be separated from mixtures in gelatin capsules with several medicinal oils and salol and determined by the U.S.P. gravimetric perchlorate method. In coated tablet mixtures

TABLE 1.—*Results on methylene blue by volumetric chlorine-perchlorate method*

WT. OF SAMPLE	PRODUCT	WT. OF $KClO_4$	HNO_3 OR OTHER R	RECOVERY	END POINT OF TITRATION
gram		*gram*	*ml.*	*per cent*	
0.5	U.S.P. of known purity	1	0	99.3	Satisfactory
0.5	U.S.P. of known purity	1	1	99.5	Satisfactory
0.5	U.S.P. of known purity	1	1	99.2	Satisfactory
0.5	U.S.P. of known purity	1	3	99.2	Satisfactory
5.0	coated tablet mixture containing 0.2103 g. of 100% m. b. anh.	1	3	99.8	Blue interfered Titrated to approx. end pt., filtered, checked end pt.
5.0	Same as above.	1	3	101.4	Same as above.
0.8839	7 pills labeled 1 gr. com'l choc. ctd. product	1	3	80.0*	Same as above.
1.0184	8 pills—Same as above.	1	3	79.3*	Same as above.
0.5	U.S.P. of known purity	1.5	3 10% alcohol	100.1	Deeper blue

* Per cent of declared, calculated to U.S.P. m. b. $3H_2O$.

containing insoluble materials such as starch, talc, calcium carbonate, the separation from insoluble ingredients was not sufficiently complete to permit a gravimetric determination. No further work was done to determine what changes in the method would be required to obtain a clean separation, and the subject was therefore recommended for continued study.

In view of the favorable results reported by Maurina and Deahl,[1] on the volumetric chlorine determination by silver nitrate after perchlorate precipitation, this method was tried on authentic mixtures (similar to coated tablets) and on a U.S.P. sample of known purity and commercial chocolate coated pills of 1 grain labeled strength. The results obtained are given in Table 1.

[1] *J. Am. Pharm. Assoc.*, 32, 301 (1943).

The end point in the case of back titration of the U.S.P. product is satisfactory with thiocyanate, but it is not satisfactory when applied to tablet mixtures. Further study would be required to improve this part of the method.

Difficulty was experienced in maintaining constant oven temperatures at 110°C. ±2° for the moisture determination on the authentic sample for this work. This led to trials in the vacuum oven at lower temperatures. The results of three determinations at 70°–80°C. in 5–8 hours averaged 15.15 per cent, compared with 14.89 per cent in the air oven as specified by the U.S.P. for an 18-hour drying period. This is a major problem in connection with the determination of this compound. It has been established that decomposition occurs from heating at or above 110°C., and when dried at this temperature the dried product may not be used for assay purposes. Therefore, it seems advisable to reconsider vacuum drying, when these objections probably would be eliminated. There seems to be a good prospect also of shortening the drying period.

It is recommended* that the subject be continued.

REPORT ON ETHYLAMINOBENZOATE AND ITS OINTMENT

By H. W. CONROY (Food and Drug Administration, Federal Security Agency, Kansas City, Mo.), *Associate Referee*

In accordance with recommendations made last year, investigational work on benzocaine and its ointment was undertaken. These products are described in U.S.P. XII, but no assay is provided. The ointment is composed of 5 parts of benzocaine to 95 parts of an ointment base of the following composition: 90 parts white petrolatum, 5 parts white wax, and 5 parts wool fat.

The following procedures were tried to determine the most suitable method: (1) *Gravimetric.*—Very good results were obtained when the extracted benzocaine was dried over sulfuric acid, but evidence of loss on drying at 80°C. was noted. (2) *Alkaline hydrolysis, followed by titration of excess alkali* (*C.A.*, **36**, 3002 (1942)).—Results were about 0.5 per cent high by this procedure. (3) *Two bromination procedures.*—(a) Method of Fiyalkov and Yampolska;[1] (b) method proposed by E. H. Wells (*This Journal*, **24**, 737). Method (b) gave slightly better results and was easier to manipulate. This procedure was used in the collaborative work.

Of the available methods, the Wells bromometric procedure was found to be most suitable for the estimation of benzocaine. After separation of the compound from the base, this procedure was also used for the assay of benzocaine in ointment.

* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 48 (1945).

[1] *Arch. Pharm.*, **270**, 203 (1932); *C. A.*, **26**, 3329, 4911 (1932).

For the purpose of collaborative study, a sample of benzocaine complying with U.S.P. XII purity tests and a 5 per cent benzocaine ointment of U.S.P. XII composition were submitted to collaborators to be analyzed; the method will be published in the 6th edition of *Methods of Analysis*, 1945.

TABLE 1.—*Results of collaborators*

COLLABORATOR*	BENZOCAINE	BENZOCAINE IN OINTMENT
	per cent	*per cent*
T. C. Dunn	102.3	5.07
	102.4	5.12
		5.12†
		5.08†
G. E. Keppel	100.0	5.04
	100.1	5.05
		5.09†
S. H. Perlmutter	100.0	5.06
	100.1	5.07
		5.07†
M. E. Warren	100.5	5.07
	100.5	5.07
	100.7	5.10†
		5.10
		5.02†
F. D. Roach	100.1	5.03
	100.0	5.05
	100.3	5.07
	99.6	
F. J. McNall	100.1	5.13
	100.0	5.13
		5.11†
H. P. Bennett	100.1	5.01†
L. Jones	100.0	5.06
		5.07
Associate Referee	99.6	4.96
	99.8	5.03
Average	100.3	5.06

* All collaborators are members of the U. S. Food and Drug Administration.
† Cellophane was used to weigh and introduce sample into separator.

COMMENTS OF COLLABORATORS

H. P. Bennett.—I suggest the use of a tared piece of cellophane as a means of weighing and introducing the ointment into the separator.

M. E. Warren.—Weighing this sample of ointment direct into a small beaker or by difference from the metal tube was found to be quite as convenient as weighing on cellophane and inserting in the separator.

The hydrochloric acid used in extracting from the ointment is designated "5% HCl" in the directions for assay. This is the reagent containing 50 ml. of concentrated hydrochloric acid per liter, and is of course not 5% HCl. It might eliminate confusion to refer to the reagent in another manner.

In all determinations four extractions were found sufficient to remove all the benzocaine. The procedure is easy to use and not time consuming.

DISCUSSION

A correction in the designation of the hydrochloric acid of 50 ml. per liter concentration from "5% HCl" to "1+19 HCl" was incorporated in the method. The suggestion that cellophane be used to weigh and introduce the sample into the separator was tried by most of the collaborators, and the results compare favorably with results obtained when the sample was weighed in a beaker or from the metal tube.

The average recovery of benzocaine from the ointment is 101.2 per cent. No brominating substance was extractable from the ointment base by the method. The Associate Referee considers that the collaborative results are in sufficiently close agreement and that the recovery is such as to warrant the use of the method.

It is recommended* that the outlined procedure be adopted as a tentative method for the assay of benzocaine and its ointment.

No reports were given by the Associate Referees on the following subjects: metrazol, barbiturates, acetanilid, sulfanilamide derivatives, phenolphthalein in presence of bile salts, atabrine (chinacrin).

REPORT ON SEDORMID IN TABLETS

By Iman Schurman (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

In a preliminary report (*This Journal*, **27**, 357) a method was proposed for the determination of sedormid in a tablet mixture, which depended upon the hydrolysis of sedormid to urea with further hydrolysis of the urea to ammonia. This method gave quantitative results. In the same report it was pointed out that the sedormid might be extracted from the dry mixture with chloroform and determined quantitatively by evaporation of the solvent. This method was also found to give good results.

* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 48 (1945). The details of the method will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

Upon recommendation of Committee B (*This Journal*, **27**, 53), which questioned the necessity of so elaborate a method as the urea method, collaborative study along this line was discontinued.

Accordingly this year the work was confined to the extraction method, and samples were submitted for collaborative study.

Preliminary experiments carried out with pure sedormid showed that the product is slightly volatile at 100°C. and especially so if dried in a forced-air type oven. A weighed amount of sedormid was placed in a tared beaker, 100 ml. of chloroform was added and the solvent was evaporated on a steam bath. A few ml. of ether was added and again evaporated to apparent dryness. The beaker was placed in a forced-air oven at 100°C. for 10 minutes. After cooling in a desiccator the beaker was weighed. The weight of the residue was equal to the weight of sedormid. Repeating the drying and weighing for 2–10 minute periods the per cent recovery was 99.89 and 99.54, respectively. After a total heating for 2 hours, the recovery dropped to 98.13 per cent. In a Freas oven the volatility is almost negligible.

Sample No. 1 consisted of sedormid tablets, labeled 4 grains each. The average net weight of 100 tablets is 6.58 grains. On the basis of the label declaration the per cent sedormid in the tablet is 60.79. The tablets were ground to pass an 80-mesh sieve. Samples of the ground material were submitted to the collaborators.

Sample No. 2, a synthetic mixture containing 65 per cent sedormid and 35 per cent starch, was also submitted to the collaborators.

The collaborative results are shown in the table.

COMMENTS

Collaborator No. 4.—It is suggested the method incorporate an additional 10 minutes' drying period for constant weight since these samples showed a loss of 0.1 mg.–1.0 mg. after second heating.

Collaborator No. 5.—After six extractions, test for complete extraction was made.

Collaborator No. 6.—It is suggested that the directions be more specific. At least 8 or 10 extractions were made with small amounts of chloroform in order to complete extraction. Perhaps I should have used a larger volume of the solvent.

DISCUSSION OF RESULTS

The results on Sample No. 1 calculated to grains per tablet agree quite closely among themselves and within tolerance of the declared amount. Results on Sample No. 2 are also in good agreement and agree reasonably well with the theoretical value. The results of collaborator No. 5 were uniformly lower on both samples.

The melting points where reported corrected are also satisfactory. It appears that the melting point taken with a Fisher melting point apparatus are uniformly a little higher.

TABLE 1.—*Collaborative results*

SAMPLE NO.	1		2		1	2	REMARKS
COLLABORATOR	SEDORMID	CALCULATED	SEDORMID	RECOVERY AVERAGE	MELTING POINT		
	per cent	*grams/tablet*	*per cent*	*per cent*	°C.		
1	58.9	3.88	64.5	99.2	195–6	195–6	Corrected
	59.0	3.88	64.5				
2	59.1	3.89	64.1	99.0	195–6	195–6	Corrected
	59.1	3.89	64.3				
3	59.05	3.89	—		194		None
	59.06	3.89	—				
	59.11	3.89	—				
4	59.2	3.90	64.6	99.4	196.5	198	Uncorrected
	59.2	3.90	64.6		197	198	Fisher M.P. Apparatus
5	57.7	3.80	62.4	97.0	198	196	Uncorrected
	58.0	3.82	63.5				
6	59.23	3.90	63.55	97.4	197	197	Uncorrected
	58.65	3.86	63.05		197	197	
7	59.66	3.93	64.30	99.0	196–7	196–7	Fisher M.P. Apparatus
8	59.69	3.93	65.23	100.4	195–7	195–7	Fisher M.P. Apparatus
	59.91	3.94	65.28				
Associate	59.57	3.92	64.84		195–6	195–6	Corrected
Referee	59.87	3.94	64.90	99.8			
	59.81	3.94	64.85		196–7	196–7	Fisher M.P. Apparatus
Average	59.15	3.89	64.28	98.9			

RECOMMENDATIONS*

It is recommended—

(1) That the chloroform extraction method be adopted as a tentative method for the determination of sedormid in tablets.

(2) That the melting point of 194–197°C. be accepted.

The Associate Referee wishes to express his thanks to the following collaborators for their wholehearted cooperation: Mr. Conroy, Mr. Bennett, Mr. Carson, Mr. Horwitz, Miss Segall, Mr. Berry, Mr. McNall, and Mr. Fine, all members of the Food and Drug Administration.

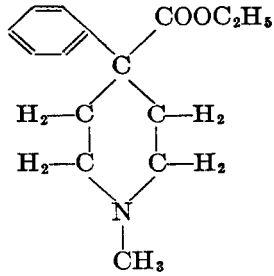
* For report of Subcommittee B and action by the Association see *This Journal*, 28, 48 (1945). Details of the method will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON DEMEROL

By MERLIN MUNDELL (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In accordance with a recommendation by the Referee on Synthetic Drugs, a study of methods for the determination of Demerol was initiated. Demerol is a synthetic drug which is reported to have analgesic and antispasmodic properties. It is being sold as a substitute for morphine. It was synthesized in 1939 by Eisleb and Schaumann[1] and was introduced in Germany under the name of Dolantin. This drug was recently classified by Congress as a narcotic drug within the meaning of the Narcotic Drugs Import and Export Act. Because Demerol has been trademarked as a brand name and should not be employed in legislation, the name "isonipecaine" was coined as a suitable designation. In the Act, isonipecaine was defined to mean "any substance identified as 1-methyl-4-phenyl-piperidine-4-carboxylic acid ethyl ester, or any salt thereof, by whatever trade name designated."

Demerol has the structural formula:



It is a white crystalline substance that melts at 30°C. and is only slightly soluble in water. Its solutions are strongly alkaline. For medicinal purposes, the hydrochloride is used. It also is a white crystalline powder that melts at 187–188° and dissolves readily in water.

Lehman and Aitken[2] and Oberst[3] have reported methods for the determination of demerol in urine. Their methods, which were designed to determine the amount of the drug present in very weak solutions, do not appear to be suitable for the determination of demerol in tablets.

Schoen[4] reported that ephedrine can be determined by steam distilling it from strongly alkaline solutions followed by acidimetric titration of the distillate. Since the free base of demerol melts at 30°, it was thought that it might be determined by distillation from a weakly alkaline solution followed by titration. It seemed probable also that demerol could be determined by an extraction procedure similar to the method for ephedrine

[1] *Deut. med. Wochschr.*, 65, 967–968.
[2] *J. Lab. Clin. Med.*, 28, 787 (1943).
[3] *J. Pharmacol.*, 79, 10 (1943).
[4] Schoen, *J. Am. Pharm. Assoc.*, 33, 116–118.

sulfate which is described in the United States Pharmacopoeia.[5] Both methods were tried by the Associate Referee and seemed to give satisfactory results.

DISTILLATION METHOD

APPARATUS

An all-glass apparatus was assembled, using pieces made with standard taper (24/40) ground glass points. It consisted of a 500 cc. round bottom, short neck flask into which fitted an adapter with attached separatory funnel (Ace catalog, 5270). A distilling head (Scientific Glass Apparatus Co., J-1500) fitted into this adapter. A connecting adapter (Ace, 5125) was used to connect the distilling head to a straight inner-tube, water cooled condenser. Another adapter, attached to the bottom of the condenser, was long enough to dip below the surface of the liquid in a 1,000 cc. Erlenmeyer flask. Other apparatus could be set up which would probably work equally well. The essential features are provision for adding liquid during the distillation and a distilling head efficient enough to prevent any spray of the calcium carbonate mixture from carrying over into the receiving flask.

REAGENTS

Methyl red indicator (*Methods of Analysis*, 5th edition, **XXXIX**, 82(b), N/50 NaOH standardized against sulfamic acid[6] using methyl red indicator. N/50 H_2SO_4 standardized against the N/50 NaOH using methyl red indicator.

Powdered calcium carbonate.

PREPARATION OF SAMPLE

Weigh a counted number of not less than 20 tablets and reduce them to a fine powder without appreciable loss.

DETERMINATION

Weigh accurately an amount of the powder equivalent to ca. 100 mg. of Demerol. Wash it into a 500 cc. round bottom flask with ca. 25 ml. of water. Introduce into flask ca. 1 gm. of powdered $CaCO_3$ and connect the flask to the distillation apparatus described above. Place 1-liter Erlenmeyer flask, containing 20 ml. of N/50 H_2SO_4 under condenser in such manner that the adapter on end of condenser will be below surface of the acid. Thru separatory funnel introduce into flask 100 cc. of water. Heat until only ca. 25 cc. of liquid remains in flask. Without interrupting distillation, add a second 100 cc. of water slowly enough that distillate does not suck back into distillation flask. Continue distillation until this portion has distilled over. In like manner, distill over a third 100 cc. of water. Then add, thru the separatory funnel, 10 cc. of ethyl alcohol. When most of alcohol has distilled over, add and distill over a fourth 10 cc. portion of water. Disconnect condenser from distillation apparatus and rinse inside of condenser and adapter which dipped into the standard acid, catching rinsings in receiving flask. Bring collected distillate to vigorous boil to remove any dissolved CO_2, cool, and titrate excess acid with N/50 NaOH, using methyl red as indicator. Each ml. of N/50 H_2SO_4 = .005673 gm. of demerol hydrochloride $C_{15}H_{21}O_2N \cdot HCl$.

EXTRACTION METHOD

REAGENTS

Methyl red indicator. N/50 H_2SO_4. N/50 NaOH. Approximately 1 *N* H_2SO_4. Approximately 1 *N* NaOH.

Sodium chloride.

PREPARATION OF SAMPLE

Same as for distillation method.

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[5] *Pharmacopoeia of the United States*, Twelfth Edition, p. 466.
[6] Butler, Smith, and Andrietti, *Ind. Eng. Chem., Anal. Ed.*, 10, 690 (1938).

DETERMINATION

Weigh accurately a portion of powder, equivalent to ca. 0.1 gm. of Demerol, and macerate it with 10 cc. of distilled water and 1 cc. of normal H_2SO_4 for 2 hours. Decant liquid through small filter into separatory funnel. Macerate residue with 5 cc. of distilled water for 20 minutes, filter thru same filter and wash residue and filter with small portions of distilled water. Saturate the soln with NaCl, then add 5 cc. of normal NaOH and extract with 25 cc. of ether. Draw off aqueous layer into another separator, and repeat extraction of aqueous layer in similar manner 6 times, using 20 cc. of ether each time. Wash combined ether extracts with two 5 cc. portions of distilled water, then extract this water with 10 cc. of ether and add this ether to main ether extract. Extract ether soln first with 20 ml. of N/50 H_2SO_4, accurately measured, then successively with 10 cc. and 5 cc. of distilled water. Combine the H_2SO_4 and water extracts in beaker and warm on water bath until odor of ether is no longer perceptible. Cool soln and titrate excess acid with N/50 NaOH, using methyl red indicator. Each ml. of N/50 H_2SO_4 is equivalent to .005673 gm. of demerol hydrochloride, $C_{15}H_{21}O_2N \cdot HCl$.

RESULTS

No collaborative work has been done on these methods, but the Associate Referee has been able to get good recoveries with samples of pure demerol hydrochloride* and with samples which were a mixture of demerol hydrochloride, starch, lactose, and stearic acid made up to simulate commercial tablets. The results are shown in the tables on the next page.

DISCUSSION

In the distillation method, it seemed advisable to distill from a weakly alkaline solution because demerol is an ester which would probably be hydrolyzed in the presence of strong alkali. At first, a buffer solution of *p*H 7.5 was used which gave good results with the pure drug. With tablet mixtures, the sodium and potassium salts in the buffer reacted with the stearic acid in the tablets to form soaps which caused excessive foaming. It was found that the substitution of a calcium carbonate suspension in water for the buffer solution overcame the difficulty of foaming by forming an insoluble calcium soap and also made the solution alkaline enough to permit the distillation of the Demerol.

The Demerol under these conditions distilled very slowly, so that it was necessary to collect a fairly large volume of distillate. Alcohol was added toward the end of the distillation to wash out any of the Demerol adhering to the side walls of the connecting tube and condenser. Slightly lower results were obtained if the alcohol was not used.

The values obtained by the extraction method were approximately the same as those obtained by the distillation method. Slightly high values were obtained with samples of the pure drug, which suggests that some sodium hydroxide was carried along in the ether extract. This was verified by making a blank determination. Enough alkali was found in the ether to neutralize 0.13 ml. of N/50 sulfuric acid, which is equivalent to 0.74 mg.

* The Associate Referee is grateful to the Alba Pharmaceutical Co., Inc., for furnishing the above sample.

TABLE 1.—*Distillation Method*

SAMPLE	DEMEROL HCl IN SAMPLE	DEMEROL HCl RECOVERED	PER CENT RECOVERED
Pure Demerol HCl	100.5	100.4	99.9
	100.7	99.9	99.2
		Av.	99.55
Tablet Mixture	100.8	100.6	99.8
	99.7	99.2	99.5
	100.9	100.9	100.0
		Av.	99.8

TABLE 2.—*Extraction Method*

SAMPLE	DEMEROL HCl IN SAMPLE	DEMEROL HCl RECOVERED	PER CENT RECOVERED
Pure Demerol HCl	100.7	102.4	101.7
	100.2	100.2	100.0
		Av.	100.85
Tablet Mixture	101.2	100.8	99.6
	100.2	99.4	99.1
	100.0	99.2	99.2
	100.5	99.6	99.1
	100.7	99.7	99.0
		Av.	99.2

of Demerol. The lower recoveries obtained with tablet mixtures were probably due to incomplete extraction of the drug from the tablet mixture.

Both methods seem to give approximately the same recoveries when used with tablet mixtures. The distillation method, however, seems to be preferable because of the slight balancing error in the extraction method. The distillation method has another advantage in that it is probably more specific.

RECOMMENDATIONS*

It is recommended that the topic be continued in order that these methods may be subjected to collaborative study.

No reports were given by the Associate Referees on the following subjects: propadrine hydrochloride, carbromal, phenolsulfonphthalein.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 48 (1945).

REPORT ON PROCAINE

By JOSEPH LEVINE (Bureau of Narcotics Laboratory, U. S. Treasury Department, Washington, D. C.), *Associate Referee*

Three methods for the quantitative determination of procaine, two of them official and one tentative, have been adopted by the A.O.A.C.[1]

In Method I, which is bromometric, the *p*-aminobenzoic acid produced on hydrolysis of procaine is treated with excess standard bromide-bromate solution, and the excess is determined volumetrically. In Method II the free procaine base obtained by extraction from an alkaline solution of the sample is determined by titration with standard acid. In Method III (tentative) the alkaline distillate obtained on hydrolysis of procaine is determined by titration with standard acid.

BROMOMETRIC METHOD

In a recent study of bromination procedures such as that applied in Method I, Wells[2] reported that in order to obtain quantitative results with procaine a longer period of bromination than that designated in the method was necessary. In three analyses he reported values of from 93.4 per cent to 96.5 per cent, with an average recovery of 95.4 per cent. Quantitative results were obtained only when either the period of bromination or the excess of bromide-bromate solution was increased. It was recommended that the time of bromination be increased from the half hour designated in the method to two hours.

Accordingly, the official Method I was studied. Ten samples of U.S.P. procaine hydrochloride were assayed with strict adherence to the time intervals of the several steps of the procedure as described. A recovery of 99.7 per cent $\pm$ 0.15 per cent was obtained (Table 1). A recovery of 100.2 per cent was obtained for two samples in which the bromination period was lengthened to two hours, and an average of 100.6 per cent was obtained for three samples in which a three-hour bromination period was allowed.

The values obtained in the analyses fail to confirm Well's findings in regard to the inadequacy of the bromination period designated in the official method. Increase in the length of the bromination period did result in somewhat higher analytical values, but quantitative results are obtained by the method as described. No reason for the low results reported by Wells has been suggested.

DISTILLATION METHOD

Method III (tentative) is based upon the procedure of Matchett and Levine.[3] It depends upon the ready hydrolysis of procaine to yield an alka-

[1] *Methods of Analysis, A.O.A.C.*, 1940, 590, 97–99.
[2] *This Journal*, 25, 537 (1942).
[3] *Ibid.*, 23, 58 (1940).

line product, diethylamino ethanol, which may be quantitatively steam-distilled and then titrated. This method is applicable to samples of procaine admixed with materials other than salts of volatile amines or compounds that are readily hydrolyzed to yield volatile amines. Procaine is dispensed in the form of tablets, with inert excipients or with epinephrine, and as solutions with sodium chloride, sodium sulfite, epinephrine,

TABLE 1.—*Results obtained by Method I with varying bromination periods*

TIME OF BROMINATION	WEIGHT	0.1 *N* BROMATE CONSUMED	RECOVERY
hours	*gram*	*ml.*	*percent*
½	0.0949	20.85	99.8
½	0.1030	22.60	99.6
½	0.1149	25.22	99.7
½	0.1096	24.17	100.1
½	0.1024	22.56	100.0
½	0.0994	21.77	99.5
½	0.1003	22.00	99.6
½	0.1002	21.95	99.5
½	0.1075	23.60	99.7
½	0.0965	21.12	99.6
2	0.1020	22.50	100.2
2	0.1070	23.60	100.2
3	0.1110	24.60	100.6
3	0.1140	25.30	100.7
3	0.1106	24.50	100.6

or ephedrine.[4] In the illicit traffic, procaine has appeared as an adulterant in mixtures of heroin or cocaine, together with cane or milk sugar.

Method III is directly applicable to all of these preparations, except the solution with ephedrine. Although epinephrine has a structure similar to ephedrine, it does not steam-distil nor is it hydrolyzed to form a volatile amine under the conditions of the assay.[5] The method is therefore applicable to procaine-epinephrine mixtures.

A collaborative study was made of Method III. Reports were submitted by six collaborators who analyzed U.S.P. procaine hydrochloride, and by two collaborators who analyzed commercial solutions or tablets. Table 2 records the results of the former, and Tables 3 and 4 those of the latter.

The overall average is 99.8 ± 0.36 per cent, with a standard deviation, σ, of 0.46 per cent.

A variation in the procedure was introduced by Collaborator G, who connected the flask containing the sample to a steam-distillation apparatus, collecting 150–200 ml. of distillate. He stated that when only 100 ml. of distillate was collected results 2–3 per cent low were obtained.

[4] "New and Non-official Remedies," 1943.
[5] Schoen, *J. Am. Pharm. Assoc.*, **33**, 116 (1944).

TABLE 2.—*Collaborative results obtained with Method III on U.S.P. procaine hydrochloride*

ANALYST	WT. SAMPLE	ADDED SUBSTANCE	0.1 *N* ACID	RECOVERY	AVERAGE	AVERAGE DEVIATION
	gram		*ml.*	*per cent*	*per cent*	*per cent*
A	1.0165	—	37.15	99.7		
	0.7489	—	27.50	100.2		
	0.7493	—	27.40	99.8		
	0.7703	—	28.41	100.6		
	0.8140	—	30.00	100.5	100.2	±0.32
B	0.9000	—	33.00	100.0		
	0.9000	—	32.95	99.9		
	1.2000	—	43.85	99.7		
	0.5000	—	18.40	100.4		
	1.0000	—	36.60	99.8	100.0	±0.20
C	0.8006	—	29.05	99.0		
	0.3000	—	10.85	98.7		
	0.3004	—	10.98	99.7		
	0.3000	—	10.94	99.5		
	0.8000	—	29.11	99.3	99.2	±0.32
D	0.5005	—	18.32	99.8		
	0.5300	Lactose	19.45	100.1		
	0.4924	Heroin hydrochloride	18.08	100.2		
	0.6545	Morphine sulfate	23.96	99.9		
	0.4916	Cocaine hydrochloride	18.01	99.9	100.0	±0.14
E	0.5009	—	18.40	100.2		
	0.4669	—	17.15	100.2		
	0.4604	Codeine sulfate	16.90	100.1		
	0.5037	Morphine sulfate	18.50	100.2		
	0.5367	Cocaine hydrochloride	19.75	100.4	100.2	±0.06
F	0.3214	—		99.2		
	0.5763	—		99.7		
	0.0500*	—		99.1		
	0.1000*	—		99.6	99.4	±0.25

* Parnas-Wagner type micro-apparatus used. 30 ml. of steam distillate collected.

Two of the collaborators stated that slightly low results were obtained when the distillation was stopped after 100 ml. had been collected, and they suggest that the distillation be continued beyond this point to collect as much distillate as possible. It is therefore recommended that the volume of distillate specified in Method III be increased to approximately 125 ml.

It is recommended* that the status of Method III (tentative) for pro-

* For report of Subcommittee B and action by the Association see *This Journal*, 28, 48, 84 (1945). The details of the method will be published in the 6th edition, *Methods of Analysis*, *A.O.A.C.*, 1945.

TABLE 3.—*Results obtained by Collaborator G*

COMMERCIAL AMPULES OF PROCAINE HYDROCHLORIDE		COMMERCIAL AMPULES OF PROCAINE HYDROCHLORIDE WITH EPINEPHERINE	
2%	1%	2%	1%
per cent	*per cent*	*per cent*	*per cent*
1.96	1.00	1.98	1.00
2.05	0.98	2.00	1.04
2.02	1.02	2.02	1.04
2.03	1.00	1.99	1.00
2.02	1.03	2.08	0.98
2.05	0.97	2.00	1.05
2.05	1.03	2.02	1.04
2.07	1.01	2 00	0.98
2.04	1.01	1.96	——
1.99	1.05	2.02	Av. 1.02
2.02	——	1.99	±0.026%
2.05	Av. 1.01	2.08	
——	±0.018%	1.98	
Av. 2.03		2.02	
±0.022%		——	
		Av. 2.01	
		±0.025%	

TABLE 4.—*Results obtained by Collaborator H*

SAMPLE ANALYZED	NO. TABLETS PER SAMPLE	TOTAL PROCAINE FOUND	PROCAINE/TABLET	
			LABELED	FOUND
		gram	*gram*	*gram*
Novocain-Suprarenin	15	0.291	0.02	0.0194
	15	0.293	0.02	0.0195
	15	0.293	0.02	0.0195
Novocain-Boric Acid Sodium Chloride	2	0.402	0.20	0.201
	2	0.394	0.20	0.197
	3	0.590	0.20	0.197
	3	0.601	0.20	0.200

caine in presence of chlorobutanol, cocaine, codeine, epinephrine, heroin, lactose, and morphine be advanced to official, first action.

No report on sulfobromophthalein was given by the Associate Referee.

MODIFICATION OF THE U.S.P. ASSAY FOR SPIRIT OF CAMPHOR

By HARRY W. CONROY (U. S. Food and Drug Administration, Federal Security Agency, Kansas City, Mo.)

The U.S.P. method for the determination of camphor in spirit of camphor by precipitation as the 2, 4-dinitrophenylhydrazine has been found to yield unsatisfactory results. The following modification permits of a better recovery of camphor with less contamination of the hydrazine by the precipitating reagent.

METHOD

REAGENTS

(1) *2, 4-dinitrophenylhydrazine.*—Dissolve 2 grams of 2, 4-dinitrophenylhydrazine in 20 ml. of 1+1 cold H_2SO_4 by shaking in a glass-stoppered flask; add 35 ml. of water, mix, cool, and filter.

(2) *Aldehyde-free alcohol.*—U.S.P. XII, p. 649.

PROCEDURE

Dilute an accurately measured quantity of spirit of camphor with aldehyde-free alcohol so that the solution contains 0.2–0.25 gram of camphor per 10 ml. To 10 ml. of the dilution in an approximately 125 ml. pressure bottle, add 50 ml. of the freshly prepared 2, 4-dinitrophenylhydrazine solution. Close the pressure bottle and immerse in water to near its top in a tall-form beaker. Heat on the steam bath 4 hours, maintaining the temperature of the reaction bottle at about 75°C., and invert the bottle 3 or 4 times during the heating period. Cool the bottle and contents to room temperature, open, and pour the contents into a beaker. Wash the precipitate remaining in the bottle into the beaker with 100 ml. of 1+11 H_2SO_4. Collect the precipitate on a tared Gooch crucible. (Filtration may be made after mixing or the solution may be allowed to stand overnight.) Wash the precipitate with 10 ml. of 1+11 H_2SO_4 and then with about 75 ml. of cold water added in small portions to remove the acid. Dry at 100°C. The weight of precipitate×0.458 = weight of camphor in 10 ml. of the dilution.

TABLE 1.—*Assay of camphor*

TYPE OF ASSAY	TYPE OF CAMPHOR	
	NATURAL	SYNTHETIC
	per cent theory	
U.S.P. XI	89.25	—
	90.06	—
U.S.P. XII	92.9	—
	91.1	—
	92.5	—
U.S.P. XII modified	99.0	99.4
	98.8	99.1
	99.1	99.1

RESULTS

In Table 1 are shown results obtained on a sample of natural camphor, purified by sublimation, and on one of synthetic camphor purified by crystallization from alcohol with subsequent sublimation.

DISCUSSION

The U.S.P. method has been criticized because of the deterioration of the dinitrophenylhydrazine reagent during the reaction period, and on standing overnight. The amount of precipitation, in a blank determination by the U.S.P. method, was found to be 12.5 mg. A smaller blank was obtained by the modified procedure, the average of three blank determinations being 1 mg. The melting points of the precipitated hydrazines agree with those given in the literature for synthetic and natural camphor.

REPORT ON MISCELLANEOUS DRUGS

By CHRIS K. GLYCART (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Referee*

Reports from four associate referees were received this year as follows:

Spectrophotometric Methods, Determination of Chrysarobin, by J. Carol.

This excellent paper is the result of original work by the Associate Referee. The absorption spectra of seven samples of chrysarobin from different commercial sources were studied and comparisons were made with controls of pure chrysophanic acid and chrysophanol, synthesized in the laboratory. The results of analysis indicate that chrysarobin has nearly the same composition and a method is now available for the assay of chrysarobin based on a definite chemical standard of chrysophanic acid.

The Referee concurs that the method be submitted for collaborative study.

Microchemical Tests for Alkaloids and Synthetics, by George L. Keenan.

The Associate Referee studied tests for quinacrine, totaquine, and sodium diphenylhydantoin. He concluded that the tests for totaquine might be postponed and caffeine be substituted for collaborative study since he found a test for caffeine more significant than the mercuric chloride test listed in *Methods of Analysis*, 5th edition, 1940, page 630.

The Associate Referee recommends that the study include quinacrine, caffeine, and diphenylhydantoin for collaborative study. The Referee concurs. The Referee also recommends that tests for butacaine and pentothal sodium be considered for future study.

Report on Emulsions by Harold O'Keefe.

The Associate Referee submitted a review of his two years' work on the

method for the determination of phenolphthalein in emulsions of liquid petrolatum with phenolphthalein. The Referee recommends that the now tentative iodination method for the determination of phenolphthalein in emulsions be advanced to official adoption and the subject be closed.

Report on Compound Ointment of Benzoic Acid, by William F. Kunke.

The Associate Referee has performed considerable experimental work and has devised a method for the quantitative determination of benzoic and salicylic acids in ointments. He found that benzoic acid did not interfere with the determination of salicylic acid by bromination and the recovery was satisfactory.

The Referee concurs that the method be adopted as tentative without further study and that the subject be closed.

Mercury Compounds (Ethanolamine Method) (P. S. Jorgensen).

No work was performed by the Associate Referee, but he has indicated that he will continue the subject next year and submit the Rotondaro modification of the Ethanolamine Method to collaborative study. The Referee concurs.

No reports were received from the Associate Referees on topics as follows:

Separation of Bromides, Chlorides, and Iodides (N. E. Freeman). The Referee recommends that the subject be continued.

Alkali Metals (W. C. Woodfin). No report was made, because of continued illness of the Associate Referee. It is recommended that the subject be continued.

Thyroid (F. A. Rotondaro). The Referee recommends that the subject be discontinued and that organic iodides be studied instead.

Glycols and Related Compounds. The Referee recommends that the subject be continued.

Preservatives and Bacteriostatics in Ampul Solutions. The Referee recommends that the subject be continued.

For next year's work, it is recommended that chromatographic methods for the separation and identification of Vitamins A, D, Thiamine Chloride, Riboflavin, Nicotinic Acid, and Nicotinamid be studied.

REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS

By GEORGE L. KEENAN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Under the direction of the Associate Referee collaborative studies already have been made and reports published on microchemical tests for the following substances: physostigmine, sodium sulfapyridine (*This*

NOTE.—For report of Subcommittee B and action by the Association, see *This Journal*, 28, 51 (1945).

Journal, 24, 830, 1941), sulfathiazole, benzedrine sulfate, metrazol (*Ibid.*, 25, 830, 1942), and choline and sulfadiazine (*Ibid.*, 26, 96, 1943). It is recommended* that these tests be advanced to official, first action.

The report submitted last year (1943) recommended that the work for the ensuing year should include a study of microchemical tests for quinacrine, totaquine, and sodium diphenylhydantoin. The Associate Referee has done considerable work on these substances and has developed tests that appear to be satisfactory for quinacrine and sodium diphenylhydantoin. However, some difficulty was experienced with totaquine and it was considered advisable to drop consideration of this substance for the time being. It was planned to send out these three substances to collaborators for study, but the uncertain results with totaquine did not allow sufficient time for such a study this year. The present arrangement contemplates sending out quinacrine and sodium diphenylhydantoin, and substituting caffeine for totaquine in next year's study. Our attention previously had been called to a sensitive and characteristic test for caffeine with the formation of a caffeine-iodine compound which appears to be more distinctive than the precipitate formed with mercuric chloride. The Associate Referee has modified the test somewhat and feels that it is well worth while testing by the collaborators.

Therefore, in view of the results obtained on the microchemical tests for quinacrine, sodium diphenylhydantoin, and caffeine, it is recommended* that they be included for collaborative study the ensuing year.

No report on mercury compounds (ethanolamine methods) was given by the Associate Referee.

REPORT ON SEPARATION OF CHLORIDES, BROMIDES, AND IODIDES

By N. E. FREEMAN (Food and Drug Administration, Federal Security Agency, Atlanta, Ga.), *Associate Referee*

A limited amount of collaborative results which have not been published will be included for next year's report. The results on the samples sent out would justify the adoption of the gravimetric "Open Carius" method as a tentative method for Total Halides in the absence of Organic Halogen compounds. However, further modifications to apply to organic compounds, especially those containing iodide, have been undertaken but not completed.

It is recommended* that the subject be continued. The Referee concurs.

No report on thyroid was made by the Associate Referee.

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 51 (1945).

REPORT ON EMULSIONS

By HAROLD F. O'KEEFE (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

Emulsions as a topic for study was first assigned by the Association in 1937.[1] W. F. Funke studied various extraction procedures for the quantitative determination of cod liver oil in emulsions.[2] After collaborative work in the second year, the method proposed by Kunke was adopted as tentative.[3]

No work was done in 1939 and 1940 and the subject was then reassigned to the present Associate Referee.[4]

In the first year a method was devised for the determination of phenolphthalein in emulsion of liquid petrolatum with phenolphthalein. The separation was made by precipitating the gums with alcohol-ether mixture, removing the phenolphthalein with dilute sodium hydroxide solution, and finally adding acid in excess, filtering and weighing the precipitated phenolphthalein.[5]

The subject was continued for the second year. The separation of the gums and mineral oil from the phenolphthalein was essentially the same as during the first year; however, the phenolphthalein was determined by the iodination procedure as described in *Methods of Analysis, A.O.A.C.*, 1940. This report was made in 1943 by the Associate Referee without recommendation.[6] In view of the agreement in results obtained by seven collaborators, it was recommended by the General Referee that the iodination method for phenolphthalein be adopted as tentative. His recommendation was approved by the Committee.[7]

REPORT ON COMPOUND OINTMENT OF BENZOIC ACID (OINTMENT OF BENZOIC AND SALICYLIC ACID)

By WILLIAM F. KUNKE (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

Compound Ointment of Benzoic Acid was a National Formulary VI product, but the identical product is now official in the National Formulary VII under the more descriptive name Ointment of Benzoic and Salicylic Acid. According to the formula it should contain 12 per cent of benzoic acid and 6 per cent of salicylic acid incorporated in a white petrolatum and wool fat base. No assay is given.

This subject has been previously studied by the Associate Referee, *This*

[1] *This Journal*, 20, 58 (1937).
[2] *Ibid.*, 21, 577 (1938).
[3] *Ibid.*, 22, 57, 86, 739 (1939).
[4] *Ibid.*, 23, 61 (1940).
[5] *Ibid.*, 25, 843 (1942).
[6] *Ibid.*, 26, 312 (1943).
[7] *Ibid.*, 27, 54, 359 (1944).

Journal, 24, 840 (1941), and 25, 845 (1942), but no report has been made since 1942. Subcommittee B recommended that the subject be continued.[1]

A brief review of the previous reports is given. Considerable investigational work has been done including (1) a critical experimental study of the conditions of bromination of salicylic acid necessary for accurate quantitative results, (2) the selective oxidation of salicylic acid in the presence of benzoic acid, and (3) the separation of the two acids from a carefully prepared ointment of known composition and their determination.

The bromination of salicylic acid was studied because the literature is surprisingly meager in giving thorough and critical investigations, and because a certain highly recommended quantitative method yielded results which were 5 per cent too low in some cases.

More than 100 experiments were made in which the quantity of salicylic acid (0.0460 gram) and the total volume of the reacting mixture (100 ml.) were constant, but the acidity, excess of bromine, and period of reaction were varied. Of these experiments, 20 determinations yielded results varying from 99.8 to 100.2 per cent and showed conclusively that the optimum conditions are (1) 25 per cent excess of bromine, (2) 5 ml. of hydrochloric acid, and (3) 30 minute reaction period. Relatively small deviations from these conditions should give equally accurate results, but wide deviations will cause poor results; as examples, a 100 per cent excess of bromine or a 2½ hour reaction period (18 experiments) yielded results which were as much as 1 per cent and 1.7 per cent too high, respectively; and a high acidity, 20 ml. of hydrochloric acid (4 experiments) caused results to be from 2.7 to 7.6 per cent too low.

It was definitely shown that benzoic acid does not consume bromine under the proposed conditions of determining salicylic acid by bromination.

Although the experiments on selective oxidation of salicylic acid in the presence of benzoic acid showed promise, it appears advisable to discontinue the attempt to determine benzoic acid directly. The benzoic and salicylic acid used in all experiments was found by the U.S.P. assay, titration with 0.1 *N* sodium hydroxide, to have a purity of 99.8 per cent.

A tentative method is proposed at this time for the determination of benzoic and salicylic acid in this ointment, although no collaborative work has been undertaken.

The method is based upon well-known and sound procedures; the extraction of both acids and subsequent titration with 0.1 *N* sodium hydroxide. Also the details have been carefully worked out for the determination of salicylic acid by bromination (benzoic acid does not consume bromine) and the benzoic acid is calculated by difference. Very good results have been obtained by the Associate Referee on a batch of carefully

[1] *This Journal*, 25, 58 (1942); 28, 51 (1945).

prepared ointment of known benzoic and salicylic acid content. As a check the weight of the acids, benzoic and salicylic, was taken before titration with 0.1 *N* sodium hydroxide and the results agreed with those obtained by the proposed method.

PROPOSED METHOD FOR OINTMENT OF BENZOIC AND SALICYLIC ACID

Transfer an accurately weighed sample, ca. 2.5 gram, into a separatory funnel, add ca. 50 ml. of ether and swirl until dissolved. Completely extract with saturated soln of $NaHCO_3$, using 15, 15, 10, and 10 ml. portions or more. Extract the combined $NaHCO_3$ soln with 10 ml. of $CHCl_3$ and discard the latter. Acidify with HCl and extract with $CHCl_3$ ether (2+1) until the benzoic and salicylic acids are completely extracted. Filter extracts into a 250 ml. beaker thru filter moistened with $CHCl_3$. Evaporate to ca. 5 ml. on a steam bath using a current of air, then continue spontaneously.

Dissolve residue in about 20 ml. of diluted alcohol (about 50%), carefully titrate with 0.1 *N* NaOH using phenolphthalein, record the volume and add an excess of ca. 2 ml. Completely evaporate the alcohol on a steam bath using a current of air; evaporation from ca. 50 ml. volume to 5 or 10 ml. is sufficient. (Alcohol consumes bromine.)

Transfer remaining titration liquid and washings to 100 ml. volumetric flask, cool to room temperature, and fill to mark with water. Mix thoroly. Pipet a 25 ml. aliquot into an iodine flask, add 25 ml. of water, exactly 25 ml. of 0.1 *N* $KBr\text{-}KBrO_3$, and ca. 5 ml. of HCl. Swirl mixture repeatedly during 30 min. Carefully add 5ml. of KI soln (ca. 10%), shake well and in about one min. titrate with 0.1 *N* $Na_2S_2O_3$ using starch indicator.

Calculate salicylic acid from the 0.1 *N* $KBr\text{-}KBrO_3$ consumed, 1 ml. = 0.0023 gram. Calculate benzoic acid from the difference between the 0.1 *N* NaOH titration value and the 0.1 *N* NaOH equivalent of the salicylic acid found in sample taken. 1 ml. of 0.1 *N* NaOH = 0.01221 gram of benzoic acid or 0.01381 gram of salicylic acid.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method be adopted as tentative.

(2) That the subject be closed.

No report on alkali metals was made by the Associate Referee.

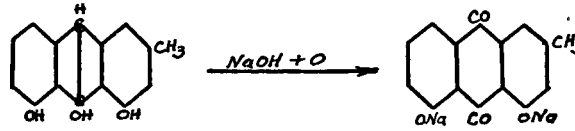
REPORT ON SPECTROPHOTOMETRIC METHODS (DETERMINATION OF CHRYSAROBIN)

By J. CAROL (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

Chrysarobin has been used for many years in the treatment of skin diseases. It is usually dispensed as an ointment or in collodion solution. Much research work has been done on the isolation and identification of

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 51 (1941).

its constituents.[1,2,3] Gardner[4] reports that chrysarobin is composed almost entirely of derivatives of chrysophanic acid and emodin monomethyl ether. However, no satisfactory methods for the analysis of chrysarobin preparations have been published. Glycart in an unpublished report describes the colorimetric analysis of chrysarobin by the red color produced in alkaline solution. It is based on the reaction by which chrysophanol, the major constituent of chrysarobin, is oxidized by air in alkaline solution to produce sodium chrysophanate.



Sodium chrysophanate forms deep red solutions suitable for spectrophotometric determinations.

The present experimental work was originally undertaken to develop a spectrophotometric method for the determination of chrysarobin in medicinal preparations based on this reaction. In the course of this work it was found desirable to study the absorption spectra of a number of chrysarobin samples, chrysophanol, and chrysophanic acid. By means of these absorption spectra it was hoped to establish that chrysarobin from different sources and different dates of manufacture are essentially the same and are composed chiefly of chrysophanol and chrysophanic acid.

EXPERIMENTAL

APPARATUS AND MATERIALS

A Beckman Model D quartz spectrophotometer fitted with matched 1 cm. cells was used.

Chrysarobin USP. Commercial samples all from different sources:

Sample	*Approximate Date of Manufacture*
(a)	New
(b)	1940
(c)	1940
(d)	1933
(e)	1925
(f)	New
(g)	New
(h)	New

Chrysophanic acid—Golden yellow spangles M.P. 195–195.5°C. (uc) prepared according to Gardner[5] from chrysarobin.

Chrysophanol.—Yellow leaflets M.P. 206°C. (uc) by the reduction of chrysophanic acid with tin and acetic acid.

[1] Tutin and Clewer, *Proc. Chem. Soc.*, **28**, 13 (1912).
[2] Hauser, Dissertation, Zürich, 1924.
[3] Naylor and Gardner, *J. Am. Chem. Soc.*, **53** (1931), 4114.
[4] J. H. Gardner, *J. Am. Pharm. Ass.*, **23**, 1178 (1934).
[5] *Loc. cit.*

In the following work extinction (E) measurements were read directly from the spectrophotometer and are defined by the Beer and Lambert law as:

$$E = \log I_0/I = kcl$$

where E = extinction
I_0 = intensity of light transmitted by blank
I = intensity of light transmitted by sample
k = specific extinction
c = concentration in g per liter
l = thickness of solution in cm.

The absorption spectra of chrysophanol and chrysophanic acid were determined by making extinction measurements from 320–500 mμ of chloroform solutions containing 1 mg. per 100 ml. These curves are shown in Figure 1. Each has a well-defined peak, chrysophanol at 356 mμ with $E^{1\%}_{1\,cm.} = 505$, and chrysophanic acid at 434 mμ with $E^{1\%}_{1\,cm.} = 486$. Chloro-

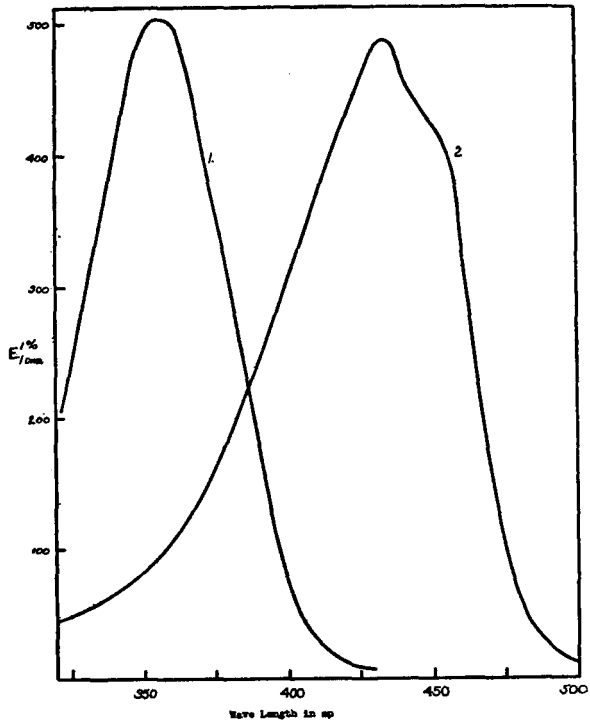


FIG. 1.—The absorption spectra of chrysophanol (1) and chrysophanic acid (2) in chloroform.

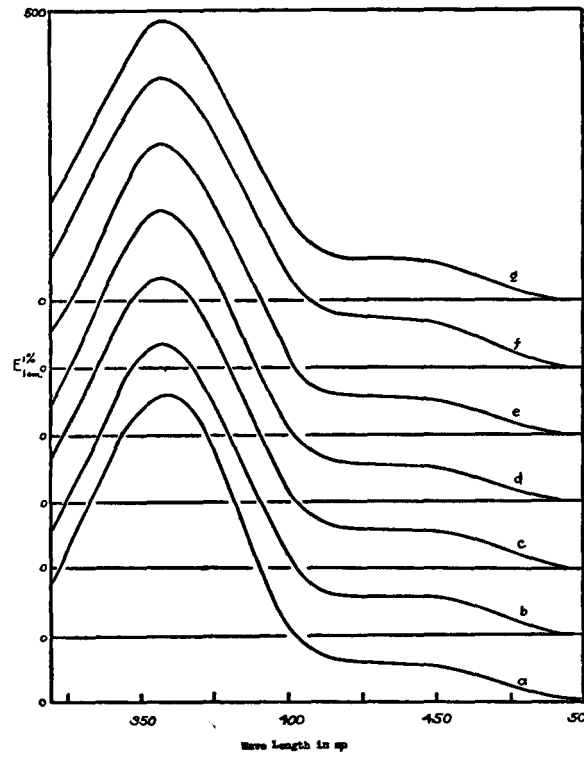


FIG. 2.—The absorption spectra of chrysarobin in chloroform (Samples a to g).

form solutions of chrysarobin containing 1 mg. per 100 ml. were prepared and their absorption spectra determined by making extinction measurements from 320–500 mμ. These spectra are plotted in Figure 2 and their $E^{1\%}_{1\,cm.}$ values at 356 and 434 mμ (the peak absorptions for chrysophanol and chrysophanic acid, respectively) are tabulated in Table 1.

TABLE 1.—*Absorption data*

SAMPLE	$E^{1\%}_{1cm.}$ AT—	
	356 mμ	434 mμ
a	455	60
b	433	60
c	430	60
d	430	57
e	430	52
f	430	68
g	417	62

In order to test the possibility of using chrysophanic acid as a standard in the spectrophotometric analysis of chrysarobin it was necessary to compare the absorption spectra of each in alkaline solution. Extinction readings were made from 320–650 mμ of the solution formed when 2 mg. chrysarobin (Sample a) was treated as directed under "Method." Extinction readings of a solution of 2 mg. chrysophanic acid per 100 ml. 0.1 *N* sodium hydroxide were also made from 320–650 mμ. These absorp-

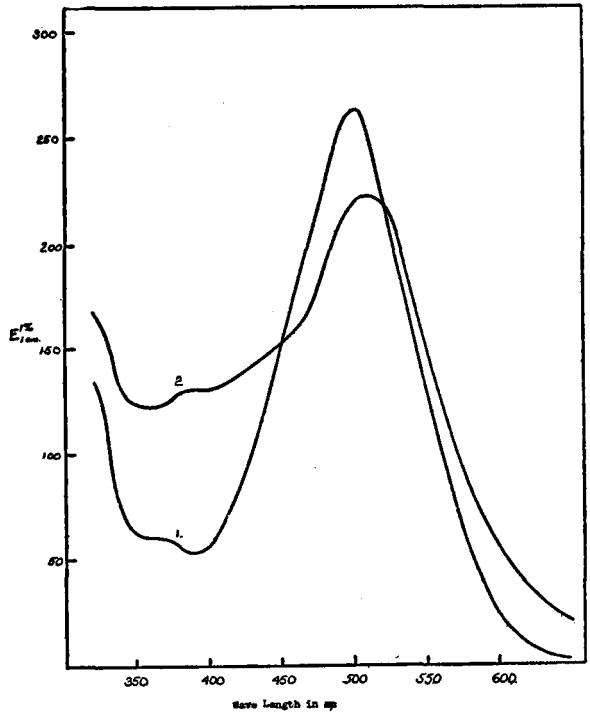


FIG. 3.—The absorption spectra of chrysophanic acid (1) and chrysarobin (2) in 0.1 *N* NaOH. Chrysarobin solution prepared as directed in method.

tion spectra are shown in Figure 3. They have the same general shape and, while their maxima are not identical, extinction measurements made at 502 mμ should be suitable for the estimation of either chrysophanic acid or chrysarobin.

To test the applicability of the Beer-Lambert law to solutions of chrysophanic acid in 0.1 *N* sodium hydroxide, extinction measurements were made of a series of solutions containing 0.5–3.0 mg. per 100 ml. at 502 mμ. These data are recorded in Table 2.

TABLE 2.—*Extinction values*

MG CHRYSOPHANIC ACID PER 100 ML 0.1 NaOH	$E_{1\ cm.}$ 502mμ
0.5	.134
1.0	.268
1.5	.403
2.0	.533
2.5	.671
3.0	.804

The straight line produced in Figure 4 by plotting extinction values against concentration demonstrates that the Beer-Lambert law is satisfied within the limits of experimental error.

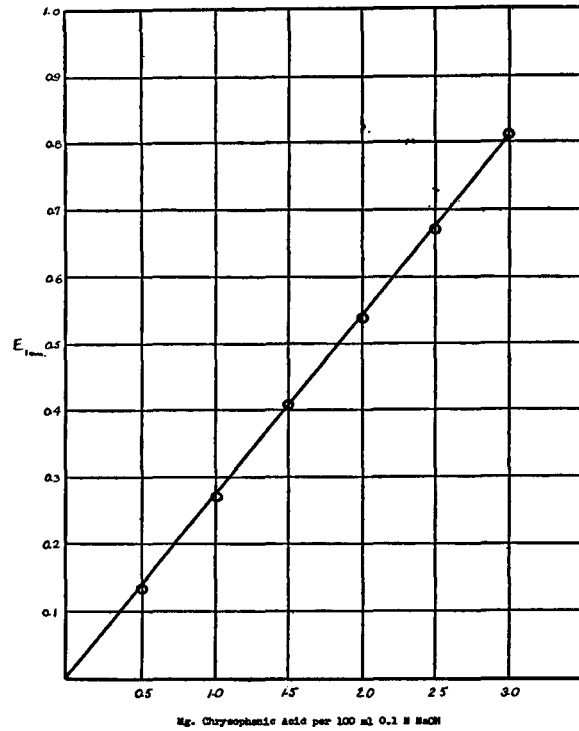


FIG. 4.—Plot of extinction against concentration at 502 mμ.

Based on the above experimental work the following method for the determination of chrysophanic acid (and derivatives) in chrysarobin and chrysarobin preparations is proposed.

METHOD

APPARATUS

A spectrophotometer suitable for measuring absorption at 502 mμ. Two matched 1 cm. absorption cells.

REAGENTS

Sodium hydroxide solution, 10% w/v.

Ether.—U.S.P. grade.

Chrysophanic acid.—Prepare according to Gardner.[5]

STANDARD SOLUTION

Dissolve 20 mg. chrysophanic acid in 25 ml. sodium hydroxide solution and dilute to 1000 ml. Solution is stable for about 10 hours.

DETERMINATION

Accurately weigh or measure a quantity of sample containing ca. 2 mg. of chrysarobin and transfer to small separatory funnel. Dissolve in 25 ml. of ether. Extract with successive 10 ml. portions of sodium hydroxide soln until aqueous layer is no longer colored after vigorous shaking. Combine aqueous extracts in 100 ml. volumetric flask and fill to mark. Determine extinction (E_{sample}) at 502 mμ using a blank of sodium hydroxide solution of approximately same concentration as sample soln. In the same manner determine extinction ($E_{standard}$) at 502 mμ of standard soln.

$$\text{Mg chrysophanic acid (and derivatives)} = \frac{E_{sample}}{E_{standard}} \times 2.0.$$

In Table 3 are shown the results of analysis of the 7 samples of chrysarobin by the proposed method.

TABLE 3.—*Analytical results*

SAMPLE	CHRYSOPHANIC ACID (AND DERIVATIVES)
	per cent
a	85.0
b	84.7
c	84.7
d	81.5
e	81.7
f	86.4
g	81.3

SUMMARY AND RECOMMENDATIONS

The absorption spectra of the series of chrysarobin samples are closely alike both in shape and magnitude of extinction. When considered in conjunction with the absorption spectra of chrysophanol and chrysophanic acid it is possible to conclude that chrysarobin from different sources and dates of manufacture are nearly the same in composition, being chiefly chrysophanol (and derivatives) with a small amount of chrysophanic acid (and derivatives).

[5] *Loc. cit.*

A study of the absorption spectra of sodium chrysophanate in 0.1 *N* sodium hydroxide and that of the solution formed in the method shows that chrysarobin is largely converted to sodium chrysophanate (or substances having almost the same absorption spectra) by oxidation by air in alkaline solution. Therefore, chrysophanic acid is a suitable standard for the spectrophotometric analysis of chrysarobin.

The results of analysis of seven chrysarobin samples by the above method show a rather narrow range of 81.5 to 86.4 per cent chrysophanic acid and derivatives. With the analysis of a larger number of chrysarobin samples it would be possible to set a minimum chrysophanic acid content upon which to base the results of analysis of chrysarobin in medicinal preparations.

It is recommended* that next year the method be submitted for collaborative study.

No report was given on glycols and related compounds nor on preservatives and bacteriostatic agents in ampul solutions.

No report was given by the Referee on drug bioassays and no reports were given by the associate referees under this general subject.

REPORT ON COSMETICS AND COAL-TAR COLORS

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

Communications from the Associate Referees indicate that for the following topics insufficient data were available to justify a formal report:

Alkalies in Cuticle Removers
Arsenic in Hair Lotions
Cosmetic Creams
Cosmetic Powders
Cosmetic Skin Lotions
Depilatories
Hair Straighteners
Lead in Cosmetics
Moisture in Cosmetics
Resorcinol in Hair Lotions
Urea in Deodorants
Ether Extracts in Coal-Tar Colors
Identification of Certified Coal-Tar Colors
Intermediates in Certified Coal-Tar Colors

In all cases but one the Associate Referee recommends that the work be continued and expresses his willingness to serve again as Associate Referee. However, the Associate Referee for "Arsenic in Hair Lotions"

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 51 (1945).

wishes to be relieved of his duties, since he will no longer be doing this type of work.

The General Referee recommends that the study of "Lead in cosmetics" and "Arsenic in Hair Lotions" be discontinued for the present, and that if possible new associate referees be found for Mercury Salts in Cosmetics and Nail Cosmetics.

No report was made on Spectrophotometric Testing of Coal-Tar Colors. The Associate Referee, however, presented a study on the "Estimation of FD&C Yellow Nos. 3 and 4 in Cottonseed and Other Vegetable Oils," employing chemical methods, and reported check results by spectrophotometric measurements. This was published as a contributed paper in the preceding number of *This Journal*, page 636.

Formal reports were received on these topics:

Deodorants and Anti-perspirants
Hair Dyes and Rinses
Mascara, Eyebrow Pencils, and Eyeshadow
Pyrogallol in Hair Dyes
Acetates, Carbonates, Chlorides, and Sulfates in Certified Colors
Buffers and Solvents in Titanium Trichloride Titrations
Halogens in Halogenated Fluoresceins
Mixtures of Coal-Tar Colors for Drug and Cosmetic Use
Pure Dye, Impurities, and Substrata in Pigments
Subsidiary Dyes in Drug and Cosmetic Colors

In each case the General Referee concurs in the recommendations made by the Associate Referees.

Methods, official and tentative, ready for inclusion in the sixth edition of *Methods of Analysis* (assuming acceptance of the recommendations made at this meeting) are:

Aluminum and Zinc in Deodorants and Anti-perspirants
2,5-Diaminotoluene in Hair Dyes and Rinses
p-Phenylenediamine in Hair Dyes and Rinses
Salicylic Acid in Hair Lotions
Sulfides in Powdered Depilatories
Alizarin in Madder Lake
Halogens in Halogenated Fluoresceins
Pure Dye in D&C Green No. 5
Pure Dye in D&C Green No. 7
Pure Dye in D&C Orange No. 3
Pure Dye in D&C Orange No. 4
Pure Dye in D&C Red No. 8
Pure Dye in D&C Red No. 31
Pure Dye in D&C Red No. 39
Pure Dye in D&C Yellow No. 7

RECOMMENDATIONS*

It is recommended—

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53 (1945). Details of the methods will be published in the 6th edition, *Methods of Analysis*, A.O.A.C., 1945.

(1) That the following methods be adopted as official, final action:
Salicylic Acid in Hair Lotions;
Alizarin in Madder Lake.

(2) That the following methods be adopted as official, first action:
2,5-Diaminotoluene in hair dyes and rinses;
Pure Dye in D&C Orange No. 4;
Pure Dye in D&C Green No. 7;
Pure Dye in D&C Red No. 39.

(3) That the following method be adopted as tentative:
Aluminum and Zinc in Deodorants and Anti-perspirants.

(4) That the study of Cosmetics and Coal-Tar Colors be continued, except for the topics "Arsenic in Hair Lotions" and "Lead in Cosmetics."

(5) That the title of the topic "Pure Dye, Impurities, and Substrata in Pigments" be changed to read "Lakes and Pigments."

No reports were made by the Associate Referees on the following subjects: alkalies in cuticle removers; arsenic in hair lotions; cosmetic creams; cosmetic powders; cosmetic skin lotions.

REPORT ON DEODORANTS AND ANTI-PERSPIRANTS

By J. H. JONES (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

To determine the analytical problems most likely to be encountered in the analysis of this type of product an analytical survey of thirty-three deodorants, anti-perspirants, and cov-odorants was made. The products examined included all the brands listed as widely used in a recent survey.[1] Out of the thirty-three brands examined, thirty contained either an aluminum or zinc compound (or both) as one of the "active" ingredients. The determination of these metals was, therefore, selected as the topic for this year's study.

There are two important methods for the determination of aluminum, the classical hydrous aluminum oxide precipitation and the 8-hydroxyquinoline procedure. The latter appears to be the preferable method for the determination of aluminum in the products considered in this report. Zinc may also be determined by precipitation with 8-hydroxyquinoline. The use of this reagent for the determination of aluminum and zinc in deodorants and anti-perspirants was investigated and later studied collaboratively.

The 8-hydroxyquinoline method gives accurate results for both alumi-

[1] "Survey of Beauty," *Modern Magazines*, New York (1942).

num and zinc in the absence of interfering elements. The crystalline precipitates obtained are easily filtered and can be dried to a constant composition at a low temperature. The conversion factors are favorable. Boric acid, glycerin, and organic acids such as citric and oxalic do not interfere unless present in large amounts. The chief disadvantage of this reagent is that it gives quantitative or partial precipitates with nearly all metals except those of the alkali group. In the great majority of the samples analyzed in the above survey, however, only one metal was present. Separations, therefore, were unnecessary, and the non-selectivity of the reagent did not matter.

The only metals, precipitated by 8-hydroxyquinoline, found in the samples examined were aluminum, zinc, magnesium, calcium, and traces of iron. A review of published formulas indicates that these are the only metals likely to be found frequently in such products. Aluminum and zinc are precipitated by 8-hydroxyquinoline in buffered acid solution. Magnesium is not precipitated in acid solution but is quantitatively precipitated in ammoniacal solution. Calcium may be partially precipitated in ammoniacal solution when the above metals are present. Aluminum or zinc may, therefore, be separated from magnesium or calcium by precipitation with 8-hydroxyquinoline in acid solution. Co-precipitation of magnesium with aluminum or zinc may, however, occur even in acid solution. The extent of the co-precipitation of magnesium with zinc depends upon the *p*H of the solution and the relative concentration of the two metals.[2] The Associate Referee's experiments indicate that if less than one mole of magnesium per mole of zinc (or aluminum) is present the co-precipitation at a *p*H of 5 is slight. If the *p*H is 5.5 or above the co-precipitation of magnesium with these elements is significant even at lower concentration ratios. The study of Moyer and Remington[2] points to similar conclusions.

The lowest *p*H at which aluminum may be [substantially] quantitatively precipitated by 8-hydroxyquinoline is about 4.9. Zinc may be quantitatively determined at a *p*H as low as 4.6. To minimize the co-precipitation of magnesium the proposed directions were designed to give a final *p*H of about 5. A re-precipitation is, however, required if a large amount of magnesium is present. Two procedures, therefore, are given for the determination of aluminum or zinc. Procedure (a) is used when, as is usually the case for deodorants and anti-perspirants, no interfering elements are present. Procedure (b) is used if considerable magnesium is present.

COLLABORATIVE WORK

Two samples were prepared and submitted to collaborators for analysis by the proposed procedures. The composition and method of preparation of the samples is given below.

[2] Moyer, H. V., and Remington, W. J., *Ind. Eng. Chem., Anal. Ed.*, 10, 212 (1938).

SAMPLE NO. 1

Aluminum (Bureau Standards Sample 44c)	2.3412	grams
Borax	2.0	grams
Glycerin	10.0	grams
Hydrochloric acid and water to make	2000.0	ml.

Preparation: The aluminum was dissolved in hydrochloric acid and the borax and glycerin were dissolved in water. The two solutions were combined and diluted with water to exactly 2000 ml.

SAMPLE NO. 2

Zinc oxide (Ignited C.P.)	5.349	grams
Magnesium oxide, C.P.	10.0	grams
Calcium carbonate, C.P.	10.0	grams
Hydrochloric acid and water to make	2000.0	ml.

Preparation: The solids were dissolved in hydrochloric acid and the solution diluted with water to exactly 2000 ml.

Sample No. 1 contains no interfering elements and the collaborators were requested to analyze it by procedure (a). The collaborators were instructed to analyze Sample No. 2 by procedure (b).

Analytical results were submitted by the following collaborators:

John Casey, Bristol-Meyers Co., Hillside, New Jersey
H. Hilfer, Lehn & Fink Products Corp., Bloomfield, New Jersey
L. A. Huard, Food & Drug Administration, Washington, D. C.
George McClellan, Food & Drug Administration, Houston, Texas
J. C. Molitor, Food & Drug Administration, Baltimore, Maryland

Their results, together with those of the Associate Referee, are listed in Tables 1 and 2 in the order that they were received.

The results of five of the six analysts on the aluminum sample (Table 1) are in excellent agreement. The greatest single deviation from the average for their twenty determinations is 0.4 per cent. The sixth analyst's results are about 1 per cent below the average. This deviation appears to be due to the lower *p*H of the solution from which the metal was precipitated. The directions for analysis sent to the collaborators stated that the procedure was designed to give a final *p*H of 4.9–5.1 but did not call for rejection of the results if this *p*H was not obtained. More specific instructions for the *p*H control have been added to the procedures given herewith.

The average recovery of aluminum by procedure (a) is slightly above 99 per cent. A higher recovery may be obtained by precipitation with 8-hydroxyquinoline in ammoniacal solution but when the latter method is used a prior quantitative separation from magnesium is required. The disadvantages of such a separation would appear to definitely out-weigh the increase in accuracy obtainable.

Since zinc is quantitatively precipitated by 8-hydroxyquinoline at a

TABLE 1.—*Collaborative determination of aluminum*

ANALYST	SAMPLE ALIQUOT	WEIGHT PRECIPITATE	*p*H FOUND	ALUMINUM	
				FOUND	CALCULATED
	ml.	*gram*		*mg/100 ml.*	*mg/100 ml.*
(1)	10	0.1977	4.9	116.1	117.1
	10	0.1972		115.8	
	20	0.3961	5.0	116.3	
	20	0.3953		116.1	
(2)	10	0.1982		116.4	
	10	0.1984		116.5	
	20	0.3951		116.0	
	20	0.3965		116.4	
(3)	10	0.1979	4.85	116.2	
	10	0.1980		116.3	
	20	0.3948	4.85	115.9	
	20	0.3951		116.0	
(4)	10	0.1958	4.3	115.0	
	10	0.1958	4.4	115.0	
	20	0.3902	4.5	114.6	
	20	0.3904	4.5	114.6	
(5)	10	0.1982	4.9	116.4	
	10	0.1981		116.3	
	20	0.3969	5.0	116.5	
	20	0.3969		116.5	
(6)	10	0.1979		116.2	
	10	0.1978		116.2	
	20	0.3958		116.2	
	20	0.3959		116.3	
			Average	116.0	

*p*H of 4.6–9.3[2] procedure (a) will undoubtedly be satisfactory for this metal also.

The average recovery of zinc by procedure (b) (Table 2) appears to be satisfactory. The individual analyst's averages vary from 98.8 to 100.5 per cent of the calculated amount. The precision for this sample is not as high as might be desired but the largest deviations from the calculated value are +0.8 and −1.4 per cent. It should also be noted that the ratio of magnesium to zinc in the collaborative sample is much higher than would ordinarily be encountered.

Since the recovery of aluminum by procedure (a) is slightly low, results

[2] *Loc. cit.*

TABLE 2.—*Collaborative determination of zinc*

ANALYST	SAMPLE ALIQUOT	WEIGHT PRECIPITATE	pH	ZINC	
				FOUND	CALCULATED
	ml.	*gram*		*mg./100 ml.*	*mg./100 ml.:*
(1)	10	0.1168		215.9	
	10	0.1163	5.0	215.0	214.9
	20	0.2323	5.1	214.8	
	20	0.2313		213.7	
(2)	20	0.2338		216.1	
	20	0.2322		214.7	
(3)	10	0.1166	5.1	215.4	
	10	0.1163	5.1	215.6	
	20	0.2330	5.1	215.0	
(4)	10	0.1146		211.9	
	10	0.1146		211.9	
	10	0.1150		212.6	
	20	0.2307		213.2	
	20	0.2298		212.5	
	20	0.2307		213.2	
(5)	10	0.1170	5.1	216.3	
	10	0.1172		216.7	
	20	0.2330	5.0	215.4	
	20	0.2332		215.6	
(6)	10	0.1156		213.7	
	10	0.1154		213.4	
	10*	0.1159		214.3	
	20	0.2302		212.8	
	20*	0.2332		215.6	
	20*	0.2332		215.6	
			Average	214.4	

* Dried to "constant weight," total time of drying two hours. Other determinations this collaborator dried overnight.

for aluminum by the re-precipitation procedure will probably be 1–2 per cent low. The precision should, however, be as good for aluminum as for zinc.

The collaborative work reported here was confined to the analysis of solutions. The procedures used are, of course, directly applicable to liquid preparations. If the product is a powder the above procedures may usually be applied to the "acid soluble" portion of the material. Extraction with hot dilute hydrochloric acid has been used successfully for the separation of aluminum and zinc compounds from creams. A collaborative study of

a complete procedure which includes a preliminary separation from the cream base is contemplated.

The procedures given in this report do not permit the determination of both aluminum and zinc in the same sample. This topic will be studied later.

RECOMMENDATIONS*

The Associate Referee recommends that the proposed method for aluminum or zinc be adopted as tentative and that the study of deodorants and anti-perspirants be continued.

No report on depilatories was given by the Associate Referee.

REPORT ON HAIR DYES AND RINSES

By J. H. JONES (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

This report presents primarily the results of a collaborative study of the determination of 2,5-diaminotoluene by an extraction procedure.

Shupe[1] in his first (A.O.A.C.) report on hair dyes outlined a general extraction procedure for the analysis of preparations in which the "active" ingredients were phenols and amino compounds. In this procedure, diamines are separated from aminophenols by extraction of the diamines with ether from a strongly basic solution. In his last report on this topic, Shupe[2] gives the results of a collaborative study of the extraction procedure as applied to 2,5-diaminotoluene. The results obtained indicated that the proposed directions were not sufficiently specific and detailed to permit all the collaborators to secure satisfactory results.

The extraction method is applicable to most diamines. The extracted diamine is recovered as the stable diacetyl derivative and this compound may therefore be used to identify the amine.[1] For these reasons a restudy of the proposed extraction method seemed desirable to establish a technique which would produce consistently satisfactory results. The results of the Associate Referee's experiments and the comments of the previous collaborators were used as a basis for the revision of the directions for analysis.

COLLABORATIVE WORK

A sample of 2,5-diaminotoluene dihydrochloride was submitted to collaborators who were requested to prepare a solution of the salt and analyze it by the proposed procedure.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] *This Journal*, 24, 871 (1941).

[2] *Ibid.*, 26, 116 (1943).

TABLE 1.—*Analysis of collaborative sample of 2,5-diaminotoluene dihydrochloride*

DETERMINATION	FOUND	CALCULATED
	per cent	*per cent*
Chlorine	36.5	36.35
Nitrogen	14.1	14.36
2,5-Diaminotoluene[a]	61.6	62.6

[a] Dichlorimide titration procedure; see reference (2).

TABLE 2.—*Collaborative analyses of 2,5-diaminotoluene dihydrochloride*

ANALYST	SAMPLE WEIGHT	DIACETYL DERIVATIVE RECOVERED	2,5-DIAMINOTOLUENE	
			FOUND	CALCULATED
	gram	*gram*	*per cent*	*per cent*
*Erwin H. Berry,	0.1000	0.1049	62.1	62.6
Chicago, Ill.	0.1000	0.1067	63.2	(pure compound)
Chandler Holt,	0.2000	0.2110	62.45	
Bourjois Mfg. Corp.,	0.2000	0.2098	62.10	
Rochester, N.Y.	0.2000	0.2105	62.30	
	0.2000	0.2114	62.55	
*George M. Johnson,	0.1930	0.2035	62.4	
St. Louis, Mo.	0.3860	0.4074	62.5	
	0.3860	0.4077	62.5	
Louis Koch,	0.1000	0.1027	60.80	
H. Kohnstamm & Co.,	0.1000	0.1034	61.21	
New York, N.Y.	0.1000	0.1046	61.92	
*J. C. Molitor,	0.1000	0.1054	62.4	
Baltimore, Md.	0.1000	0.1058	62.6	
	0.2000	0.2121	62.8	
	0.2000	0.2120	62.8	
*Ruth R. Segall,	0.0501	0.0537	63.45	
Chicago, Ill.	0.0501	0.0535	63.21	
Associate Referee,	0.1000	0.1053	62.3	
6-16-44	0.2000	0.2117	62.7	
Associate Referee,	0.1500	0.1590	62.8	
9-15-44	0.1500	0.1561	61.6	
		Variation	60.80–63.45	
		Average	62.4	

* Food and Drug Administration, Federal Security Agency.

The diamine salt was obtained from a commercial source and used without purification for the collaborative tests. Analysis of the compound (see Table 1) indicated that it was reasonably pure.

The collaborative results are listed in Table 2.

The average recovery of the diamine by the proposed method appears to be satisfactory. The results are not very precise, but only six of twenty-two determinations differ from the average by more than 1 per cent and the maximum variation is 2.6 per cent.

None of the analysts reported any difficulties in the analysis of the salt by the proposed method.

The revised procedure appears to minimize the difficulties previously encountered and apparently will give consistently satisfactory results.

RECOMMENDATIONS*

No action appears to have been taken in regard to the dichlorimide titration method for 2,5-diaminotoluene previously reported.[2] Since the collaborative results indicated that this method was satisfactory, the Associate Referee recommends that it be adopted as official, first action.

The Associate Referee also recommends that the revised extraction method for 2,5-diaminotoluene presented in this report be adopted as official, first action, and that the subject of hair dyes and rinses be studied further.

No reports on hair straighteners or lead in cosmetics were given by the Associate Referees.

REPORT ON MASCARA, EYEBROW PENCILS, AND EYE SHADOW

By PAUL W. JEWEL (Max Factor & Co., 1666 N. Highland Ave., Hollywood, Calif.), *Associate Referee*

ANALYSIS OF MASCARA

The work begun by Fuller[1] has been continued. The analysis of mascara has proved so difficult that as yet no methods are ready for collaborative study. Considerable progress has been made, however, and it is expected that parts of the method will soon be ready for such study.

Mascara consists, generally, of a mixture of soap, waxes, and colors. The soaps and waxes are not easily soluble in most solvents, and in addition are actively absorbed by the colors, which makes their extraction extremely difficult.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

[1] *This Journal*, 26, 317 (1943).

[2] *Loc. cit.*

Before any system of analysis for this product can be developed, it is imperative that some quantitative method of separating the colors from the base be found. A good deal of the work during the past few months has been directed toward this end.

In order to provide material for investigation, a mascara of the following formula was prepared:

	Part
Stearic acid	270
Triethanolamine	120
Beeswax	300
Carnauba wax	50
Color (bone black)	250

This formula makes a fairly satisfactory product, from a cosmetic standpoint, and a very large percentage of the mascara sold in the United States at the present time is based on a formula very similar to this one.

In order to separate the base from the color a survey of all of the commonly available solvents was made. It was found that carbontetrachloride is the only one that will dissolve, completely, all of the base used in this preparation. However, when the completed mascara is dissolved in this solvent the pigments peptize and pass through the filter, although filtration is extremely slow. If part of the base is first extracted with alcohol or petroleum ether and the extraction finished with carbontetrachloride most of these difficulties are obviated.

PROPOSED METHOD FOR DETERMINATION OF TOTAL BASE AND TOTAL COLORS

The best method found so far, some modification of which will probably prove to be acceptable, is as follows:

Weigh ca. 3.000 grams of mascara into a 250 cc. beaker. Melt the mass on hot plate with careful heating and distribute sample uniformly over the bottom of beaker. Add 100 cc. of absolute alcohol, heat to boiling on steam bath, remove from steam bath, and allow pigments to settle. When settling is complete or nearly so, and before the waxes start to crystallize, decant into another beaker. Repeat with two additional 100-cc. portions of absolute alcohol, allow to settle, and decant as before. Evaporate alcohol solutions to dryness on steam bath. Dissolve residue in hot carbontetrachloride and filter through CS&S filter #589 into a tared dish. Extract the pigments 4 times with 50-cc. portions of boiling carbontetrachloride filtering through the same filter. Evaporate the combined extracts, dry in oven at 100°C. for 1 hour and weigh.

The results obtained by this method are somewhat erratic, varying from 71 to 74 per cent of total base, where the theory is 75. The acid number of the base should be 82–83, while that found varies from 62 to 100. This indicates a certain degree of selective absorption of base ingredients by the colors, which makes the method somewhat less than precise. It is hoped that further investigation will eliminate these difficulties.

PROPOSED METHOD FOR DETERMINATION OF LEAD AND ARSENIC

The statement of Fuller[1] that "much work remains to be done on the determination of arsenic in samples rich in organic matter such as fats and waxes" has been verified in this laboratory many times. The determination of lead under such circumstances is equally disappointing.

To part of the mascara formula referred to above sufficient lead and arsenic were added to make 10 parts per million of each and to another 20 p.p.m. each. These were analyzed by the official A.O.A.C method, using the Gutzeit method for arsenic[2] and the dithizone method for lead.[3] The same determinations were run on the colors remaining after extraction with solvent as described in the determination of total base and total color. In every case the results for both lead and arsenic were too low and bore no relationship whatever to the amounts of each added. It is hoped that further work on the extraction method will concentrate all of the arsenic and lead in the preparation in one fraction, and that accurate determinations of these elements will be made possible.

ANALYSIS OF EYEBROW PENCILS

No work has been done on these preparations. Since their composition is not too different from that of mascara it is expected that the methods applicable to one will be applicable to the other.

ANALYSIS OF EYE SHADOWS

No work has been done on these preparations as yet but they should offer no difficulty, since their composition is relatively simple.

CONCLUSIONS

It is recommended that work be continued on the analysis of mascara along the lines already begun, and that the analysis of eyebrow pencils and eye shadows be taken up as quickly as possible after the mascara analysis is ready for collaborative study.

No report was given on mercury salts in cosmetics.

No report on moisture in cosmetics was given by the Associate Referee.

No report on nail cosmetics was given.

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[1] *This Journal*, 26, 317 (1943).
[2] *Methods of Analysis*, Ch. 29, 1940, p. 390.
[3] *Ibid.*, p. 396.

REPORT ON PYROGALLOL IN HAIR DYES

By CURTIS R. JOINER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The art of hair dyeing is one of antiquity, and it has been said that proprietary hair dyes are almost "as many as the sands of the sea."[1] A fairly common dye is one which makes use of ammoniacal silver nitrate and is usually a two-solution dye.[2] The second solution may consist of an alkali sulfide or a reducing agent. The most commonly used reducing agent is a solution of pyrogallol varying in strength from 1 to 5 per cent.[2] Salts of nickel and cobalt are also used in the same manner as silver nitrate.

In early times pastes known as "rasticks," which consisted of powdered gall-nuts and iron and copper filings, were used for dyeing the hair.[3] A modern counterpart of ancient "rasticks" contains pyrogallol in solution with iron and copper salts. A reducing agent is usually added to retard the reaction between the pyrogallol and the metallic salts until the dye is applied to the hair.[4] The pyrogallol might be supplied in the form of a powder or compressed tablets which are dissolved in the solution of metallic salts immediately before the dye is to be applied.

Pyrogallol is often used mixed with henna powder and copper sulfate.[5] The powder is made into a paste just before it is to be applied to the hair.

Mitchell[6] used a colorimetric method for the determination of pyrogallol, gallic acid, and gallotannin based upon the immediate reaction of ferrous sulfate in the presence of a tartrate with pyrogallol and its derivatives to form a reddish-violet to blue-violet compound which is soluble in water, and which, unlike the ink produced by ferrous sulfate alone, is fairly stable. Mitchell developed the color in 100 ml. Nessler tubes by mixing 1 mg. of pyrogallol or gallic acid and 2 ml. of a solution containing 0.1 g. of ferrous sulfate and 0.5 g. of Rochelle salt in 100 ml. of water. He made the solution to volume and compared it directly to a similar tube containing the unknown substance by diluting the darker of the two solutions until it matched the lighter one.

Glasstone[7] pointed out that the intensity of the violet color is dependent upon the *p*H of the solution, which should be neutral or slightly alkaline. In order to obtain the proper *p*H, he added 10 ml. of a 10 per cent solution of ammonium acetate to the Nessler tubes before developing the color.

The Associate Referee, adapting Mitchell's colorimetric method, used

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[1] Redgrove, H. Stanley, and Foan, Gilbert A., *Hair-Dyes and Hair-Dyeing Chemistry and Technique* (London, 1934), p. 14.
[2] *Ibid.*, pp. 35–42.
[3] *Ibid.*, p. 44.
[4] *Ibid.*, p. 46.
[5] *Ibid.*, p. 74; Poucher, W. A., *Perfumes, Cosmetics and Soaps with Especia Reference to Synthetics* (London, 1936), Vol. III, p. 81.
[6] Mitchell, C. Ainsworth, *Analyst*, **48**, 2–15 (1923).
[7] Glasstone, S., *Analyst*, **50**, 40–53 (1925).

the neutral wedge photometer for the determination of pyrogallol in hair dyes after a preliminary isolation of the pyrogallol by ether extraction. In selecting the proper filter to use for the determination, the color was developed and readings were made with all filters available. A list of readings obtained with several filters on a solution containing 5 mg. of pyrogallol follows:

Filter No.:	*Photometer Readings:*
46	5.81
49	7.27
51	8.20
54	8.68
56	9.29
58	8.51
65	6.90

These readings show that filter No. 56 is the most suitable one available; it was, therefore, selected for the determination.

Because of the relatively large percentages of pyrogallol found in hair dyes (up to 5 per cent), it seemed desirable to increase the range of concentration of pyrogallol over that used by Mitchell. Therefore, the concentration of Mitchell's ferrous tartrate reagent was doubled, and the color was developed with varying amounts of pyrogallol using 5 ml. of the reagent. Under these conditions, a straight line was not obtained when the photometer scale readings made with filter No. 56 and the one-half inch cell were plotted against concentrations of pyrogallol; but when 10 ml. of ferrous tartrate reagent was used, a straight line was obtained. With these conditions the maximum concentration of pyrogallol that could be read in the photometer was 7 mg. in 100 ml. A few readings are listed below, made with varying volumes of the reagent and with two different concentrations of pyrogallol. These data show how the photometer readings vary with different amounts of ferrous tartrate reagent. Since a straight line was obtained with 10 ml. of reagent, this volume was selected as the amount to be used.

Volume of Reagent:	*Photometer Readings:*	
	5 mg./100 ml.:	*7 mg./100 ml.:*
5.00 ml.	9.00	10.21
7.50	9.28	12.45
10.00	9.27	12.70
12.50	9.11	12.52

Since maximum color development is obtained in slightly alkaline solution, the proper buffer to be used had to be selected. Photometer readings obtained with the ammonium acetate solution recommended by Glasstone[7] were slightly lower than those made with an equal quantity of sodium acetate. Because of the hygroscopic nature of ammonium acetate, it is difficult to prepare solutions of a definite concentration; therefore,

sodium acetate was chosen. Photometer readings were made using 5 mg. of pyrogallol and varying volumes of a sodium acetate solution containing 15 g. per 100 ml. These readings, some of which are listed below, indicate that the color produced with 10 ml. of the solution is near the maximum that can be obtained with this concentration of sodium acetate.

Volume of Sodium Acetate Solution:	*Photometer Readings:*
5.00 ml.	8.70
7.50	9.11
10.00	9.27
12.50	9.32

After the proper conditions for the photometric determination had been chosen, there remained the problem of quantitatively extracting the pyrogallol from hair dyes. Some experimental work showed that this extraction could be made with ether in separatory funnels or in a continuous extractor. The author used two small extractors made in this laboratory, which were based on the principle of the Palkin extractor,[8] and which had a sample capacity of approximately 15 ml. Using these extractors, three hours were sufficient to remove all pyrogallol from 10 ml. of solution containing up to 6 grams per 100 ml., while quantitative recoveries were obtained with six extractions in 125-ml. separatory funnels.

Shupe[9] proposed a gravimetric method in which he extracted the acidified sample containing 1 gram of sodium bisulfite with chloroform in a separatory funnel to remove impurities, then transferred the sample to a continuous extractor and extracted the pyrogallol with ether. He next washed the ether extract with water, evaporated it to dryness and dried the residue in a desiccator to constant weight. Shupe reported recoveries ranging from 100.5 to 102.1 per cent on three aqueous solutions of pyrogallol. The Associate Referee obtained recoveries ranging from 97.5 to 101.4 per cent on three determinations with the gravimetric method, while he obtained recoveries between 99 and 101 per cent by the colorimetric method. For the following reasons the author selected the colorimetric method as the one to be studied:

(1) In using Shupe's method, relatively long periods of drying in a desiccator were necessary to remove from the residue the water which dissolved in the ether. In one case more than 48 hours were required to dry the residue to constant weight.

(2) Ether soluble impurities possibly might be extracted and weighed as pyrogallol in the gravimetric method.

(3) In using the colorimetric method the extraction with chloroform to

[8] *Ind. Eng. Chem.*, 17, 612 (1925).
[9] Shupe, Irwin S., "Cosmetics and Color," No. 4, p. 36. (A private publication of the U. S. Food and Drug Administration.)

remove impurities should be unnecessary, unless the hair dye contains chlorophyll.

(4) The colorimetric method gave somewhat better precision than the gravimetric method in the hands of the Associate Referee.

EXPERIMENTAL

Precision of Photometer Readings.—Thirty-nine different aliquots from standard solutions were used to develop the color as instructed in the method for preparing the standard curve. These determinations were made on five different days, and the photometer reading for each one was arrived at by taking the average of ten different settings of the photometer. The results are summarized in Table 1. A statistical treatment shows that there is very good agreement among the readings.

TABLE 1.—*Reproducibility of photometer readings*

CONCN. OF PYROGALLOL	NO. OF DETERMINATIONS	PHOTOMETER READINGS		PROBABLE ERROR OF A SINGLE DETERMINATION	
		RANGE	MEAN		PYROGALLOL
mg./100 ml.		*cm.*	*cm.*	*cm.*	*per cent*
2.50	16	5.00–5.10	5.063	±0.025	±0.57
5.00	23	9.40–9.48	9.445	±0.014	±0.16

Several separate standardizations were made at intervals over a period of about six months. Straight lines were obtained having a slope of one cm. photometer scale reading equivalent to 0.570 ± 0.002 mg. of pyrogallol. Although the photometer readings for the different standardizations during this period varied slightly, the net readings, obtained by subtracting the zero point of the curve from the gross readings, always checked within the limits of experimental error. These small variations in gross readings could have been caused by slight changes in the zero setting of the photometer.

Stability of Color.—The color was developed in four solutions ranging in concentration from 2.00 mg. per 100 ml. to 7.00 mg. per 100 ml. Photometer readings were made on each solution as soon as the color was developed and thereafter at intervals during 23 hours. The maximum changes in color during this interval ranged from a decrease of 8 per cent in apparent pyrogallol concentration for the 2.00 mg. per 100 ml. solution to decreases of 0.9 and 0.8 per cent for the 7.00 and 6.00 mg. per 100 ml. solutions, respectively. While the change in color at the higher concentrations of pyrogallol is not large, the photometer readings should be made soon after the color is developed in order to avoid errors from this source.

Pyrogallol Recovery from the Extraction Procedures.—Recoveries obtained by the extraction procedures on aliquots of freshly prepared pyro-

gallol solutions containing sodium bisulfite are given in Table 2. The precision and accuracy of the results are excellent.

TABLE 2.—*Pygogallol recovery*

PYROGALLOL CONCENTRATION	RECOVERY		METHOD OF EXTRACTION
gms./100 ml.	*gms./100 ml.*	*per cent*	
1.000	1.004	100.4	Separatory funnels
1.000	1.000	100.0	Continuous extractor
6.000	5.99	99.8	Continuous extractor

Stability of Pyrogallol in Aqueous Solution.—The pyrogallol content of several aqueous solutions with and without added metallic salts was determined approximately four to five months after the samples were made up. The results are listed in Table 3. Solutions 1 through 3 were prepared according to published formulae for hair dyes,[1,5] while samples 7 through 10 are typical of the reducing solution of two-solution dyes. The data show that pyrogallol is decomposed over a period of time in solution with metallic salts. A reducing agent retards this decomposition. In aqueous solution pyrogallol is slowly oxidized by atmospheric oxygen. This reaction is retarded indefinitely by sodium bisulfite.[10] The pyrogallol recoveries from the three single solution dyes varied from 0.2 per cent to 82.2 per cent, while the lowest recovery obtained from solutions 7 through 10 was 96.4 per cent. The method presented in this report is an attempt to recover only the undecomposed pyrogallol remaining in solution.

Interferences.—Price[11] concluded that two hydroxyl groups in the ortho position on the benzene ring are necessary for a compound to give the violet color with ferrous tartrate. This conclusion has been confirmed by the Associate Referee with a limited number of compounds. Phenol, salicylic acid, resorcinol, and phloroglucinol do not give the color, but catechol, gallic acid, and tannic acid do. It is possible that the latter three compounds might be found in hair dyes; in this case the melting point of the residue from an ether extraction would be sufficient to indicate the presence of some compound other than pyrogallol. A melting point determined as directed in the qualitative test, together with the development of the color in the quantitative procedure, are sufficient to confirm the presence of pyrogallol.

Liquid dyes containing chlorophyll have not been encountered by the Associate Referee; therefore, he has not checked the accuracy of the following method with this type of preparation.[12]

[1] *Loc. cit.*, pp. 39–74.
[5] *Loc. cit.*, pp. 81–91.
[10] Thorpe, Edward, *Dictionary of Applied Chemistry* (London, 1913), Vol. IV, p. 440.
[11] Price, Phyllis Honor, *Analyst*, 49, 366 (1924).
[12] The formula for a hair dye containing an infusion of henna and chamomile leaves is given in Poucher, *Perfumes, Cosmetics and Soaps with Especial Reference to Synthetics*, Vol. III, p. 81.

TABLE 3.—*Stability of pyrogallol in solution*

SAMPLE NO.	COMPOSITION PER 100 ML. OF SOLUTION		PYROGALLOL RECOVERY	
			gms./100 ml.	*per cent*
(1)	Pyrogallol	3.800 g.	0.300	7.9
	Cupric chloride	2.3 g.		
	Hydrochloric acid (1+1)	0.75 ml.		
	Sulfuric acid (1+9)	0.7 ml.		
(2)	Pyrogallol	2.000 g.	1.644	82.2
	Cobalt nitrate	10 g.		
	Sodium acid sulfite	4 g.		
(3)	Pyrogallol	2.000 g.	0.004	0.2
	Cupric chloride	4.0 g.		
	Alcohol	4.0 ml.		
(4)	Pyrogallol	1.000 g.	0.936	93.6
	Ferric chloride	0.2 g.		
(5)	Pyrogallol	1.000 g.	0.700	70.0
	Ferric chloride	0.2 g.		
	Nickel sulfate	0.2 g.		
	Cobalt chloride	0.2 g.		
	Sodium acid sulfite	2 g.		
(6)	Pyrogallol	1.000 g.	0.688	68.8
	Ferric chloride	2 g.		
	Sodium acid sulfite	2 g.		
(7)	Pyrogallol	2.000 g.	1.992	99.6
(8)	Pyrogallol	2.000 g.	1.996	99.8
	Sodium acid sulfite	4 g.		
(9)	Pyrogallol	2.000 g.	1.928	96.4
	Hydrochloric acid (1+1)	0.5 ml.	1.928	96.4
			1.972	98.6
(10)	Pyrogallol	2.000 g.	2.000	100.0
	Alcohol	25 ml.		

Samples 1 through 6 contained varying amounts of black precipitates.
Solutions 7 and 10 were dark brown but contained no precipitate.
Solution 8 was almost colorless.
Sample 9 was dark brown and contained a small amount of a dark precipitate.

METHOD

(Preparations containing chlorophyll may require additional treatment for accurate results.)

Qualitative Test.—Add 5 to 10 ml. of sample to separatory funnel containing ca. 0.5 gram of $NaHSO_3$ and extract with 2 or 3 successive 30-ml. volumes of ether. Filter ether extracts through cotton and evaporate to dryness on steam bath. Dry in oven

at 95° to 100°C. for ca. 10 min.; then dry over sulfuric acid in desiccator to constant weight. Pulverize residue, mix well, and take melting point. If it does not melt between 131° and 134°C., sublime and again take melting point, which should fall within above range. Mix small portion of residue with equal quantity of sublimed pyrogallol and determine the melting point; it should not change.

PREPARATION OF STANDARD CURVE

Ferrous Tartrate Reagent.—Dissolve 1.00 gram of sodium potassium tartrate and 0.20 gram of $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. in a volumetric flask. PREPARE FRESH DAILY.

Prepare color standards as follows: To seven 100 ml. volumetric flasks add respectively 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, and 7.00 ml. of standard pyrogallol solution (USP XI or Analytical Reagent grade, 0.1000 gram/100 ml.). Pipet into flasks, one at a time, 10 ml. each of Na acetate solution (15.0 grams of $NaC_2H_3O_2 \cdot 3H_2O$/100 ml.) and ferrous tartrate reagent and make to volume with distilled water. Mix thoroughly, fill one-half inch cell and read immediately in neutral wedge photometer, using filter centering at 560 millimicrons. Obtain zero point of curve by reading solution containing 10 ml. each of ferrous tartrate reagent and Na acetate solution in 100 ml.

Draw standard curve on large scale graph paper so that amount of pyrogallol corresponding to any photometer reading can be easily read to 0.01 mg. A straight line should be obtained over entire range of concentration.

DETERMINATION

Extract 10 ml. of sample by one of following methods. (Use 5 ml. if volume of sample available is small.) In handling sample give it a minimum of exposure to air, as pyrogallol is readily oxidized.

1. *Continuous Extraction.*—Pipet sample aliquot into small continuous extractor containing ca. 0.5 gram of $NaHSO_3$. Extract with ether until pyrogallol is completely removed (3 to 7 hours, depending upon efficiency of extractor). Determine time required for each extractor under a certain set of conditions by extracting an aqueous solution of known pyrogallol content or by testing for complete extraction as follows: After the extraction is thought to be complete, remove flask containing ether and replace it with one containing fresh volume of ether and continue extraction for ca. 30 min. Reserve main ether extract for treatment as directed in 3rd par. under "Extraction in Separatory Funnels," and treat second extract as follows: Evaporate ether on steam bath to volume of ca. 5 ml. and continue evaporation without heat until odor of ether is gone. Dissolve residue in small volume of water and filter through small paper into 50 ml. volumetric flask. Wash extraction flask and paper with several small volumes of water. Dilute to volume and mix thoroughly. Pipet 25 ml. into 50 ml. volumetric flask, add 5.00 ml. each of Na acetate solution and ferrous tartrate reagent, make to volume and mix. Take photometer readings in one-half inch cell. The pyrogallol corresponding to this reading will equal total amount contained in extract. If color is too dark to read, repeat, using smaller aliquot. If pyrogallol obtained exceeds 0.5 mg. (or a significant amount in comparison to that obtained in main extract) continue extraction with fresh volume of ether until less than that amount is obtained. Add total pyrogallol found to that determined in main ether extract.

2. *Extraction in Separatory Funnels.*—Pipet sample into 125 ml. separatory funnel containing ca. 0.5 gram of $NaHSO_3$, add 50 ml. ether and shake vigorously for at least one min. Separate ether layer and filter through small piece of cotton previously wet with ether. Make 5 more extractions, using 30 ml. ether and for each

and filter each extract through same piece of cotton into one beaker or flask. Make seventh shake-out, filter ether through same piece of cotton into separate beaker and use to test for complete extraction as directed under "Continuous Extraction," beginning with, "Evaporate ether . . . ."

Occasional samples that will emulsify so badly as to make extraction in continuous extractor impossible can be successfully extracted in separatory funnels by using volume of ether equal to 5 or 6 times the volume of sample.

Evaporate the main ether extract, from either method of extraction, on steam bath to volume of ca. 5 ml. and continue evaporation without heat until odor of ether is completely gone. (It is not necessary to evaporate to dryness.) Dissolve residue in water and wash completely into 100 ml. volumetric flask. Dilute to volume and mix. If solution is not clear, filter through dry paper and discard first 20 ml. of filtrate. Pipet 5 or 10 ml. into 100 ml. volumetric flask and develop color as directed under "Preparation of Standard Curve," beginning, "Pipet into flasks . . . ." If photometer reading falls near lower end of scale, repeat, using larger aliquot; if color is too dark to read, dilute suitable aliquot (10 to 25 ml.) to 100 ml. in volumetric flask and use aliquot of diluted solution to develop color as directed above. From standard curve obtain amount of pyrogallol corresponding to photometer reading and calculate to gram/100 ml. in original sample.

COLLABORATIVE WORK

Two samples of the following compositions were sent to collaborators. Sample No. 2 was prepared from the formula for a typical black hair dye.[1] The pyrogallol used was of USP XI quality and melted at 131.5°–132.5°C. No difference could be detected between it and analytical reagent grade by the method of analysis presented in this report. The collaborators' results are listed in Table 4.

No. 1

Pyrogallol	41.000 grams
Sodium bisulfite	30 grams

Dissolved in distilled water and diluted to one liter.

No. 2

Pyrogallol	25.000 grams
Sodium bisulfite	50 grams
Nickel sulfate	100 grams
Ferric chloride	50 grams

Dissolved in distilled water and diluted to one liter.

COLLABORATORS' COMMENTS

Schurman: "No particular difficulty was encountered. The seventh extraction in Sample 1 did give a test for pyrogallol. Sample 2 was blank."

Carson: "Under the heading, Continuous Extractor, no instructions are given for handling the main ether extract. This might cause confusion with the test for complete extraction."

Miss Warren: "In using the continuous extraction method, the sample aliquot was diluted in the extractor with 15 ml. of distilled water, in order to have a sufficient

[1] *Ibid.*, p. 55.

TABLE 4.—*Collaborative results*

COLLABORATOR*	PYROGALLOL RECOVERY				METHOD OF EXTRACTION††	MELTING POINTS °C.	
	SAMPLE 1		SAMPLE 2			NO. 1	NO. 2
	g./100 ml.	*per cent*	*g./100 ml.*	*per cent*			
Iman Schurman	3.98	97.1	2.24	89.7	No. 2	132–3	132–3
	3.91†	95.4	2.26	90.5	No. 2		
William Horwitz	4.02	98.1	2.28	91.2	No. 2	132.5–3	132–3
	4.01	97.8	2.24	89.7	No. 2		
	4.09	99.8	2.34	93.7	No. 1		
N. A. Carson	4.20	102.4	2.50	100.0	No. 1	133.5	133
	4.18	102.0	2.50	100.0	No. 1		
Mary Warren	4.20	102.4	2.40	96.0	No. 1		132–3
	4.16	101.6	2.40	96.0	No. 1		
	4.12	100.4	2.64	105.6	No. 2		
	4.10	100.0	2.40	96.0	No. 2		
H. W. Conroy	4.21	102.7	2.48	99.2	No. 2	133	134
	4.19	102.2	2.48	99.2	No. 2		
L. Jones	4.18	102.0	2.48	99.2	No. 2	132	134
	4.15	101.2	2.48	99.2	No. 2		
O. L. Evenson	4.12	100.5	2.21	88.4	No. 2	130	
	4.06	99.0	2.13	85.2	No. 2		
Associate	4.11	100.2	2.40	96.0	No. 1	132–3	131–2.5
Referee	4.11	100.2	2.39	95.6	No. 1		
			2.42	96.8	No. 1		
Averages	4.122	100.5	2.384	95.4			

* Addresses in order of listing: Cincinnati, Ohio; Minneapolis, Minn.; St. Louis, Mo.; New Orleans, La.; Kansas City, Mo.; Kansas City, Mo.; Washington, D. C.; all of the U. S. Food and Drug Administration.

† Not included in the average. Statistical analysis showed that, according to the Pierce-Chauvenet criterion,[12] it could be discarded.

†† No. 1, Continuous extractor; No. 2, separatory funnels.

STATISTICAL INTERPRETATION OF RESULTS

Sample 1: Standard deviation, ±0.062 mg., or ±1.5%; probable error of a single determination, ±0.042 mg., or ±1.0%; probable error of mean, ±0.099 mg., or ±0.24%.

Sample 2: Standard deviation, ±0.122 mg., or ±5.1%; probable error of a single determination, ±0.082 mg., or ±3.3%; probable error of the mean, ±0.018 mg., or ±0.76%.

[12] Crumpler, Thomas B., and Yoe, John H., *Chemical Computation and Errors* (New York, 1940), pp. 189–190.

depth of liquid for the solvent to pass through. However, the extraction time required in the Palkin extractors used (50 ml. capacity) was much greater than three hours—five to seven hours were required. . . .

" . . . It seems to this analyst that testing by extracting an aqueous solution of known pyrogallol content would be almost mandatory if the test for complete extraction must be carried out as the instructions require. The color is developed in the entire portion of the test extraction, and if the extraction is so incomplete that the color is too dark to be read in the photometer, part of the extraction is lost and the determination is ruined. . . .

"Both samples darkened appreciably during pipeting into the extractors. However, Sample no. 2 darkened more rapidly and to a deeper hue. . . .

"The method is easy to use, and with the exception of the instructions regarding the test extraction, as commented on above, the instructions seem to be adequate."

Conroy and Jones: "When the aliquot was varied for the development of the color concordant results were not obtained. . . .

"Since the above results indicate that the presence of greater concentrations of bisulfite introduced into the solution retarded the color development, the following test was made:

"Before adding the sodium acetate and iron solutions to develop the color, about 0.02 gm. sodium bisulfite was added to a 10 cc. aliquot and about 0.20 gm. sodium bisulfite added to another 10 cc. aliquot. The aliquot containing the higher concentration of sulfite gave a lower recovery of pyrogallol as indicated below.

Sodium Bisulfite added	*Pyrogallol gms./100. cc.*	
	LJ	*HWC*
0.02 gms.	4.18	4.19
0.20 gms.	3.52	3.35 . . . . "

Evenson: "A 5-ml. aliquot of each sample was taken. Six extractions were made. For No. 1(A) the seventh extraction gave 0.35 mg. and two more extractions gave 0.6 mg. For No. 1(B) the seventh extraction gave 0.2 mg. For No. 2(A), the seventh extraction gave 0.05 mg. and for No. 2(B), 0.2 mg."

The method presented in this report is a revision of the one submitted to the collaborators. The changes were made as a result of the collaborators' comments, except for a few minor changes which the Associate Referee felt were desirable. The effect of excess sodium bisulfite on the color development, which was pointed out by Jones and Conroy, was checked by the Associate Referee with the following results: Sodium bisulfite in concentrations up to and including 0.03 gm./100 ml. in the final colored solution had no measurable effect. A concentration of 0.04 gm./100 ml. caused a loss in recovery of approximately 1 per cent, while 0.10 gm./100 ml. resulted in a loss of about 4 per cent. The original method called for the addition of about 0.2 gram of sodium bisulfite per 100 ml. to the standard solution and to all sample solutions. Therefore, if a sample aliquot larger than 15 ml. were used by the collaborators for the development of the color the results would be in error. The addition of sodium bisulfite to the standard solution and the sample solutions was omitted entirely in the revised method because the amount of oxidation of pyro-

gallol which will take place in an aqueous solution during the course of an analysis under normal conditions is negligible.

The instructions for developing the color in the tests for complete extraction were changed as suggested in Miss Warren's comments, and the wording of the instructions for handling the ether extracts was revised after consideration of Carson's comment.

SUMMARY AND RECOMMENDATIONS

Good results were obtained by the collaborators on Sample No. 1, which is representative of one of the solutions of many two-solution hair dyes.

While the results obtained on Sample No. 2, which is a typical one-solution dye, are not good, in consideration with the data given in Table 3, it is doubtful if much better results could be expected. With but one exception, the maximum spread between duplicate determinations by any one chemist was 4 per cent. Since pyrogallol is slowly decomposed in such solutions and is readily oxidized on exposure to air, it is probable that storage conditions and the method of handling the sample would affect the recovery obtained.

It is recommended* that further work be done on the applicability of this method and on the extraction of pyrogallol from solid hair dyes.

No reports were given by the Associate Referees on resorcinal in hair lotions, salicylic acid in hair lotions, and urea in deodorants.

REPORT ON ACETATES, CARBONATES, HALIDES, AND SULFATES IN CERTIFIED COAL-TAR COLORS

(SODIUM ACETATE IN FD&C BLUE NO. 1)

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

The tentative method for the determination of sodium acetate[1] in coal-tar dyes has been in use for a number of years. During this time the chief complaints registered against it have been: (1) It is time consuming; (2) it gives low results; (3) the reproducibility is not satisfactory; (4) with some batches of FD&C Blue No. 1 so much of the blue dye is carried over in the distillation that subsequent titration of the distillate is difficult.

Typical results by the tentative method are:

Reagent blank: 0.36, 0.38, 0.44 per cent; average 0.39 per cent.
Acetate in the dye: 0.45, 0.54, 0.57 per cent; average 0.52 per cent.
Recoveries of added known quantities: 76.9, 86.7, and 87.9 per cent; average 83.8 per cent.
Time required per sample: 4 hours, including the determination of the blank.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 51 (1945).
[1] *Methods of Analysis, A.O.A.C.*, 1940, 255.

An adaptation of the Freudenberg method[2] which depends on the determination of acetyl groups, has been investigated in an attempt to find a less time-consuming method, giving more accurate results and permitting greater precision.

The details of the preliminary work have already been published in *This Journal.*[3] A comparison on the same basis as that above shows:

Reagent blank: 0.20, 0.22 per cent; average 0.21 per cent.
Acetate in the dye: 0.08, 0.09, 0.10, 0.21 per cent; average 0.12 per cent.
Recoveries of added known quantities: maximum 100.0 per cent, minimum, 96.00 per cent; average (of 10) 97.63 per cent.
Time required per sample: 2 hours (including blank).

A comparison of the two methods on the basis of data thus far obtained tends to show that the modified Freudenberg method is about twice as rapid as the tentative method now in use, gives better recoveries, and greater reproducibility. The use of a trap prevents all but a trace of color from being carried over with the distillate.

It is recommended*—

(1) That the modified Freudenberg method be submitted for collaborative study; and

(2) The work on this topic be continued.

No report on alizarin in madder lake was given by Associate Referee (see report of Referee).

REPORT ON BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATION

By O. L. EVENSON (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Samples of D&C Orange No. 4, D&C Green No. 7, and D&C Red No. 39 were sent to the Calco Chemical Company, H. Kohnstamm & Company, Inc., Allied Chemical & Dye Corporation (National Aniline Division), and to R. N. Sclar and S. S. Forrest of the U. S. Food and Drug Administration, with a request that pure dye be determined by methods furnished the collaborators.

The results for pure dye are given in Table 1 in the order the reports were received.

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[2] Freudenberg, K., and Harder, M., *Ann.*, 433, 230 (1923), 494, 68 (1932).
[3] Sclar, R. N., and Clark, G. R., *This Journal*, 27, 472 (1944).
* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53 (1945).

TABLE 1.

COLOR	COLLABORATOR	PURE DYE
		per cent
	1	89.5
	2	89.9
D&C Orange No. 4	3	89.8
	4	89.9
	5	89.7
	Average	89.8
	1	78.9
	2	79.8
D&C Green No. 7	3	79.5
	4	79.6
	5	79.5
	Average	79.5
	1	98.9
	2	98.9
D&C Red No. 39	3	98.5
	4	99.7
	5	97.9
	Average	98.8

The collaborators were requested also to determine moisture in the samples by the following method:

METHOD FOR VOLATILE MATTER

Weigh ca. 2 grams of sample into weighing bottle ca. 1.5 inches in diameter and dry in air oven at 135°C. (100°C. for D&C Red No. 39), for six (6) hours or overnight. Cool in a desiccator and weigh. Repeat heating and weighing at hourly intervals until weight becomes constant (±1.0 mg.). Report loss in weight as volatile matter.

Results are shown in Table 2.

TABLE 2.—*Moisture content*

COLOR	MOISTURE CONTENT		
	AVERAGE	HIGH	LOW
	per cent	*per cent*	*per cent*
D&C Orange No. 4	4.4	5.6	3.9
D&C Green No. 7	6.0	6.8	3.9
D&C Red No. 39	0.32	0.48	0.25

COMMENTS

Calco Chemical Company.—The precision of the titanium trichloride titration expressed as the reproducibility which is to be expected ninety-five times out of a hundred, for each of these methods of analysis is as follows: For D&C Orange No. 4, ±0.14, for D&C Green No. 7, ±0.17, and for D&C Red No. 39, ±0.56 per cent.

S. S. Forrest.—The methods worked very well. Excellent checks were obtained. Considerable care must be used with D&C Orange No. 4 in order to obtain check results as the end point comes slowly. The addition of a little alcohol might be useful in this case.

DISCUSSION

The average deviation of the results obtained for percentage of pure dye is, for D&C Orange No. 4, ±0.12, for D&C Green No. 7, ±0.2, and for D&C Red No. 39, ±0.46.

RECOMMENDATIONS*

It is recommended that the methods for the determination of pure dye in D&C Orange No. 4, D&C Green No. 7, and D&C Red No. 39 be adopted as official, first action.

No report on ether extracts in coal-tar colors was given by the Associate Referee.

REPORT ON HALOGENS IN HALOGENATED FLUORESCEINS

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

The work done on this problem in the past year has been principally on the determination of bromine in brominated fluoresceins.

The method of decomposition reported by J. H. Jones in 1942[1] has been found to give satisfactory results, but since air must be passed through the system during the oxidation, the adjustment of the apparatus is rather time consuming. For this reason, another method was chosen for investigation, details of which will be published in the 6th edition of *Methods of Analysis*, 1945.

PROPOSED METHOD

The apparatus used is a simplified form of that described by Willard and Thompson.[2] The absorption flask and the upper condenser with the

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53, 37 (1945). The details of the methods will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

[1] *This Journal*, 25, 944 (1942).

[2] *J. Am. Chem. Soc.*, 52, 1893 (1930).

dropping funnel are as described by these authors. The lower condenser and flask have been replaced by a 19/38 to 10/30 standard taper adapter and a 100-ml. flask with a 19/38 standard taper joint. It is much easier to place the sample in this flask than in the combined condenser and flask described by Willard and Thompson.

The sample is oxidized by chromic oxide in approximately 50 per cent aqueous sulfuric acid solution, the liberated halogens absorbed in a basic hydrazine solution, and the bromine determined according to the method described by Lang.[3]

DISCUSSION

A sample of commercial tetrabromofluorescein was analyzed for bromine by the Associate Referee; the following results were obtained.

45.36%	45.42%	45.46%
45.62	45.39	45.35
45.31	45.50	45.39

The average of these figures is 45.42 per cent, and the standard deviation is 0.1 per cent, equal to ± 3 parts per thousand. Analyses of a sample of certified D&C Red No. 21 showed 48.61 per cent, 48.49 per cent, and 48.59 per cent of bromine present.

TABLE 1.—*Collaborative results: determination of Br in dibromofluorescein*

COLLABORATOR	BROMINE FOUND	DEVIATION FROM AVERAGE
	per cent	*per cent*
I	32.66	+0.13
	32.66	+0.13
II	32.30	−0.23
	32.60	+0.07
III	32.42	−0.11
	32.49	−0.04
IV	32.65	+0.12
Associate Referee	32.56	+0.03
	32.46	−0.07
	32.49	−0.04
	32.51	−0.02
	32.57	+0.04
Average	32.53	

[3] Lang, R., *Z. anorg. allgem. Chem.*, **144, 75** (1925); (*see also*, Clark and Jones, *This Journal*, **26, 433** (1943)).

A sample of dibromofluorescein, prepared by S. H. Newburger, was analyzed by the Associate Referee and the following collaborators: J. H. Jones, S. H. Newburger, R. N. Sclar, and F. A. Rotondaro, all of the Food and Drug Administration. The results are given in Table 1. The standard deviation calculated from these results is 0.1 per cent, equal to ±3 parts per thousand. The average of the determinations is 99.75 per cent of the theoretical figure, 32.61 per cent bromine.

REMARKS

The oxidation procedure proposed has been found to give satisfactory results with chlorine compounds, and can be used when chlorine and bromine are to be determined in the same sample according to the method described by Clark and Jones.[4]

Results so far obtained indicate that iodine, at least in iodinated fluoresceins, does not interfere with the bromine determination by the proposed method, as it is oxidized to the non-volatile iodic acid. In view of the results obtained, the proposed method appears to be of satisfactory precision.

RECOMMENDATION

It is recommended* that the proposed method be made official, first action, for the determination of bromine in brominated fluorescein dyes.

No reports on identification of certified coal-tar colors or on intermediates in certified coal-tar colors were made by the Associated Referees.

REPORT ON MIXTURES OF COAL-TAR COLORS FOR DRUG AND COSMETIC USE

ESTIMATION OF MIXTURES OF D&C RED NOS. 6 AND 7 (LITHOL RUBINE B, NA, AND CA), AND D&C RED NOS. 10–13 (LITHOL RED, NA, CA, BA, AND SR)

By W. C. BAINBRIDGE (H. Kohnstamm & Co. Inc., Brooklyn, N. Y.), *Associate Referee*

Koch[1] has shown that lithol red can be extracted with ether from an acid solution, whereas lithol rubine cannot. The experimental method as presented herewith is based on the difference in solubility.

Procedure.—Boil a 0.25 gram sample of the mixture gently until solution is effected, with 100 ml. of methyl cellosolve, methyl ether of ethylene glycol, and 5 ml.

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[4] *Loc. cit.*

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 55, 87 (1945). Details of the method will be published in the 6th edition, *Methods of Analysis, A.O.A.C.*, 1945.

[1] Koch, Louis (H. Kohnstamm & Co., Inc.), *This Journal*, 25, 948 (1942).

of HCl, in a 500 ml. wide neck Erlenmeyer flask, covered with a watch glass. Buffer the soln with 10 grams of sodium bitartrate dissolved in 75 ml. of boiling water, and titrate with 0.1 *N* $TiCl_3$, adding the titanous dropwise when approaching the end point. Calculate as ml. of titer for the two dyes.

Dissolve a 0.25 gram sample with 100 ml. of methyl cellosolve and 5 ml. of HCl, by boiling gently until solution is effected. (A 250 ml. Erlenmeyer flask and air condenser, interchangeable glass joint, is recommended.) Cool and transfer to a 500 ml. extraction funnel, washing in residual dye with 10 ml. portions of ether. Bring the ether volume up to 150 ml. and separate the miscible solution that forms, with 250 ml. of a 10 per cent salt solution. Extract the lower layer with another 75 ml. of ether.

Combine the extracts, and wash them with 30 ml. volumes of water, until the washings are colorless. Run the ether into a 500 ml. wide mouth Erlenmeyer flask, and rinse the extraction funnel with two 10 ml. volumes of ether. Evaporate the ether to dryness, being careful to avoid spattering. Dissolve the residue in 100 ml. of methyl cellosolve, heating to effect solution. Buffer with 10 grams of sodium bitartrate in 75 ml. of boiling water. Heat the mixture to boiling, and titrate with 0.1 *N* $TiCl_3$, adding the titanous dropwise when approaching the end point. Calculate as lithol red.

1 ml. of 0.1 *N* $TiCl_3$ = 0.01001 gram of lithol red Na
0.01115 " " " " Ba/2
0.009935 " " " " Ca/2
0.01053 " " " " Sr/2
0.01020 " " lithol rubine Na
0.01013 " " " " Ca/2

ml. titer for mixture − ml. titer for lithol red = ml. titer for lithol rubine.

TABLE 1.—*Results*

ADDED		FOUND		ADDED		FOUND		TOTAL DYE			
LITHOL RED		LITHOL RED		LITHOL RUBINE B		LITHOL RUBINE B		ADDED		FOUND	
mg.	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*	*mg.*	*per cent*
2.5	1.0	4.2	1.68	247.5	99.0	246.1	98.44	250	100	250.3	100.12
50.0	20.0	51.3	20.52	200.0	80.0	198.0	79.20	250	100	249.3	99.72
100.0	40.0	99.7	39.68	150.0	60.0	149.0	59.60	250	100	248.7	99.48
150.0	60.0	148.6	59.44	100.0	40.0	102.8	41.12	250	100	251.4	100.56
200.0	80.0	200.2	80.08	50.0	20.0	51.2	20.48	250	100	251.4	100.56
245.0	98.0	244.4	97.76	5.0	2.0	7.6	3.04	250	100	252.0	100.80
247.5	99.0	248.3	99.32	2.5	1.0	0.6	0.24	250	100	248.9	99.56

DISCUSSION

Koch (*This Journal*, **25**, 952), has pointed out that the isomeric dyes usually found in lithol red are not ether soluble. Hence, their presence will result in too low values for the lithol red, and too high percentages for the lithol rubine. Similarly, lower sulfonated dyes in lithol rubine will increase the titer of lithol red.

If the ether insoluble dye fraction and the water washes are concentrated to ca. 300 ml., buffered with 15 grams of sodium bitartrate, and titrated with 0.1 *N* titanium chloride, a direct estimation of the lithol

rubine content can be made. This procedure has not been used, because it did not give results that were as accurate as the percentage dye by difference. However, it is recommended that the direct titration of lithol rubine as well as the lithol red should be studied collaboratively.

This method has given statisfactory results in our own laboratory. It is recommended* that the topic be continued and the method studied collaboratively.

REPORT ON PURE DYE, IMPURITIES, AND SUBSTRATA IN PIGMENTS

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

In 1941 a method for the determination of pure dye in certain lakes was reported.[1] The lakes were dissolved in concentrated sulfuric acid, alcohol added, and then a solution of sodium hydroxide and a buffer in water. The color was titrated with titanium trichloride.

Since the lithol reds are among the most widely used lake colors, it was decided to prepare a sample of pure D&C Red No. 10, and to investigate the application of the method to this color.

A sample of a lake containing 91 percent of D&C Red No. 10 was exhaustively extracted with 95 per cent alcohol. The extracted dye was crystallized from the same solvent, and dried at 135°C. Analysis of the product according to the above titration procedure showed only 95 per cent pure dye present. The pure dye content of the sample calculated from the nitrogen found upon analysis by the semi-micro Kjeldahl procedure was 95.5 per cent.

The sulfated ash obtained from the crystallized material was 17.07 per cent. Qualitative analysis showed no metal other than sodium present. The calculated sodium content of pure D&C Red No. 10 is 5.75 per cent, while the sodium content of the sample, obtained from the sulfated ash figure, was 5.52 per cent, or 96 per cent of the theoretical figure.

A portion of the sample was then dried for three hours over phosphorous pentoxide in an Abderhalden drier held at the temperature of boiling toluene. The system was evacuated by a Hyvac pump. The loss in weight was 4.4 percent.

Titrations of the dried material according to the method described showed 99.0 per cent, 99.5 per cent, and 100.0 per cent pure dye present.

As a result of these investigations, it is concluded that the titration procedure can be expected to give satisfactory results, at least in the analysis of lithol reds.

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* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 55 (1945).

[1] Clark, G. R., "Report on Lakes and Pigments," *This Journal*, 24, 904 (1941).

It is interesting to note that the results obtained upon analysis of the crystallized sample compare very well with the values calculated for a monohydrate of the dye, as shown in the following table:

TABLE 1.—*Results on crystallized sample of D&C Red No. 10*

	FOUND	CALCULATED FOR MONOHYDRATE
	per cent	*per cent*
Nitrogen	6.65	6.70
Sodium	5.52	5.55
Volatile Matter (in vacuo)	4.4	4.3
Anhydrous Color by $TiCl_3$ Titration	95.0	95.7

It is also possible that two molecules of the color may crystallize with one molecule of alcohol, although the analytical results do not agree quite so well with this theory. No attempt has been made to prove the existence of such a hydrate or alcoholate.

Drying tests made on samples of certified lakes of D&C Red No. 10 show that they contain volatile matter that can be removed by heating in a vacuum but not by heating in an ordinary oven at 135°C. Since the properties of the substrata have not been investigated, no conclusion can be drawn as to the hydration of the dye molecules.

Louis Koch,[2] in his 1942 Report on Subsidiary Dyes in D&C colors, pointed out that the Colour Index formulas for D&C Reds No. 6 and 7 were incorrect, since both the sulfonic and carboxylic acid groups are involved in salt formation. There is no doubt that Mr. Koch is correct. The titration factors calculated from the Colour Index formulas, given in the Associate Referee's 1941 Report on Lakes and Pigments,[3] should be changed.

For D&C Red No. 6, the factor should be:

1 ml. of 0.1 *N* $TiCl_3 = 0.0107$ gram of pure dye,

and

1 gram of pure dye = 93.45 ml. of 0.1 *N* $TiCl_3$.

For D&C Red No. 7,

1 ml of 0.1 *N* $TiCl_3 = 0.0106$ gram of pure dye,

and

1 gram of pure dye = 94.34 ml. of 0.1 *N* $TiCl_3$.

It is recommended* that work on this topic be continued and that the title of the topic be changed to read "Lakes and Pigments."

No report was given on spectrophotometric testing of coal-tar colors, but a paper was presented by S. H. Newburger under the title "Estimations of FD&C Yellow Nos. 3 and 4 in Cottonseed and Other Vegetable Oils," which was published in the preceding number of *This Journal*, p. 636.

[2] *This Journal*, 25, 948 (1942).
[3] Clark, G. R., *loc. cit.*
* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 53 (1945).

REPORT ON SUBSIDIARY DYES IN D&C COLORS

By L. Koch (H. Kohnstamm & Co., Inc., Brooklyn, N. Y.),
Associate Referee

D&C Red No. 6

Two samples of D&C Red No. 6, one of which was adulterated with added subsidiary dye (p-toluene-azo-beta-naphthol-3-carboxylic acid), were submitted to six collaborators to be analyzed according to the method of the Association Referee.[1]

The results of three collaborators, Leo A. Huard, Food & Drug Administration, Washington, D. C., J. D. Nantz, National Aniline Division, Allied Chemical & Dye Corp., Buffalo, N. Y., and S. Zuckerman, H. Kohnstamm & Co., Inc., Brooklyn, N. Y., for whose valuable cooperation the author is extremely grateful, are outlined in Table 1, together with the Associate Referee's.

TABLE 1.—*Collaborative results (per cent)*

ANALYST NO.	SAMPLE 1		SAMPLE 2	
		Av.		*Av.*
1	0.09		1.87	
	0.16		1.91	1.89
	0.11	0.12		
2	0.7		2.8	
	0.4	0.55	2.5	2.65
3	0.53		1.80	
	0.18	0.36	1.98	
			1.98	1.92
4	0.32			
	0.27	0.30	2.05	
			2.25	2.15

COMMENTS BY COLLABORATORS

Leo A. Huard.—The method outlined in your report appears to be satisfactory. This collaborator experienced no difficulty in following the procedure outlined. However, since the amount of intermediate found is so small, I would suggest using 0.05 *N* or 0.02 *N* titanium trichloride for greater accuracy.

The ether extraction on the original dye solution would be easier to handle and probably more accurate if performed with two 75 ml. portions of ether instead of the prescribed single extraction with 150 ml.

J. D. Nantz.—We are not satisfied with the titanium trichloride end point and would prefer the addition of some indicating dye of greater color contrast upon reduction, such as FD&C Green No. 2, which changes from a green to an orange-red in the buffer medium.

[1] Koch, *This Journal*, 25, 948 (1942).

We also think that the amount of wash water should be roughly specified, otherwise the final volume of ether will vary considerably and, if too large, may result in incomplete extraction with the caustic solution.

S. Zuckerman.—This collaborator found, when the alkaline extract stood overnight, that the addition of the tartaric acid to the alkaline-alcohol solution of the subsidiary dye formed a precipitate which did not dissolve on boiling. However, the addition of 50 ml. of methyl cellosolve brought about complete solution.

If the acidic ether layer, containing the subsidiary dye, was washed with 50 ml. portions of water until the washings were faintly acid to congo red paper, it was found that the color was removed from the ether layer very easily with the stipulated 100 ml. of 2 per cent alkali; and ca. 5–6 water washes were found to be sufficient.

DISCUSSION

Although the small number of collaborative reports precludes a definite statement as the efficacy of the proposed method, the findings indicate that the procedure is generally sound, but could be improved by incorporating some of the collaborators' suggestions. It is possible that for quantities of subsidiary dye of less than 1 per cent, it would be desirable to substitute a colorimetric comparison against a standard solution of the subsidiary dye, or one whose shade and strength is very similar, for the titration with standard titanous.

SUMMARY AND RECOMMENDATIONS

The collaborative results for the estimation of one of the subsidiary dyes in D&C Red Nos. 6 have been presented, and it is recommended* that the study of the subject be continued.

D&C Green No. 5

The subsidiary dye content (1-p-tolylamino-4-o-sulfo-p-tolylaminoanthraquinone) of two samples of D&C Green No. 5 was analyzed collaboratively according to the procedure of the Associate Referee (as published in *This Journal*, 26, 485, 1943).

The findings of the three collaborators, and of the Associate Referee, are outlined in Table 2.

COMMENTS BY COLLABORATORS

Leo A. Huard.—This method proved to be rather difficult. On making the sample alkaline with 25 ml. of 50 per cent sodium hydroxide, a heavy precipitate sometimes appeared. No mention of this is made in the procedure. This precipitate was very troublesome and made the ensuing ether extraction difficult and inaccurate.

The extraction of the liberated amine would probably be more complete and certainly easier to handle if performed with four 50 ml. portions of ether instead of two 100 ml. portions.

J. D. Nantz.—The method states that the best results are obtained when 20 ml. or less potassium bromate-bromide is used with a 5 ml. excess of the latter. The procedure for standardization results in a consumption of 6.27 ml. potassium bromate-bromide with an excess of about 13 ml.

* For report of Subcommittee B and action by the Association, see *This Journal*, 28, 56 (1945).

Adjustment of the proper volume of sample solution means that two sets of extractions are necessary. The method should be written so that the solution after extraction will be sufficient to allow another bromination of the para-toluidine to be effected without the necessity of an additional series of extractions.

The quantity of bromine consumed by the p-toluidine is a function of time, temperature, and acid concentration. Our experience has shown that bromination at 0° C., with an excess of bromine, will produce almost a theoretical conversion of the p-toluidine to dibromo-p-toluidine in five minutes.

TABLE 2.—*Collaborative results (per cent)*

ANALYST NO.	SAMPLE 1		SAMPLE 2	
		Av.		*Av.*
1	4.14		2.54	
	2.50		2.05	
	3.56		2.30	
	3.34	—	2.40	2.32
2	5.2		2.5	
	5.3	5.25	2.5	2.5
3	5.23		2.58	
	5.38	5.31	2.59	2.59
4	5.23		2.64	
	5.24	5.24	2.51	2.58

DISCUSSION

The tentative adoption of the proposed method must be left for the future, because of the small number of collaborators reporting. In general, the procedure seems reliable, although one analyst experienced considerable difficulty.

During an investigation of the reduction of D&C Red No. 34, 2-sulfo-beta-naphthalene-azo-beta-naphthol-3-carboxylic acid, with stannous chloride, it was observed that considerable desulfonation occurred. Although it is generally true that radical exchange in the naphthalene series occurs with greater ease than in benzene compounds, the possibility of desulfonating p-toluidine-o-sulfonic acid made it imperative that the question should be verified experimentally. Accordingly, 2.5 grams of the sulfonated amine were subjected to the conditions for the determination of the mono-sulfonated dye in D&C Green No. 5, with the results shown in Table 3.

The fact that the quantity of bromine absorbed appeared to be independent of the aliquot taken, led the Associate Referee to analyze the reduction mixture of the p-toluidine-o-sulfonic acid qualitatively for the presence of primary amine. A 50 ml. aliquot of the acid solution was made alkaline and subjected to steam distillation. The distillate was diazotized,

TABLE 3.—*Results*

ALIQUOT TAKEN	0.05 *N* $KBrO_3$ CONSUMED
gm.	*ml.*
0.625	1.6
1.250	1.7
0.625	1.9
0.625	1.2

coupled to beta naphthol, and compared to 0.75 mg. of p-toluidine diazotized and coupled similarly. Whereas the latter gave a distinct orange color, the former had a dirty yellow shade which could not be attributed to the formation of an azo dye. It was therefore concluded that the consumption of potassium bromate must be attributed to another factor than the formation of a primary unsulfonated amine by the process of desulfonation.

Further experimental work finally disclosed that the consumption of potassium bromate was caused by the reagents and would necessitate a negative correction for the quantity of subsidiary dye found. There is a possibility, however, backed by a few experiments, that quantitative distillation of the primary amine, and consequent elimination of the reagent blank, is a feasible procedure.

SUMMARY AND RECOMMENDATIONS

The collaborative results for the estimation of mono-sulfonated subsidiary dye in D&C Green No. 5 have been presented, and it is recommended* that the study of the topic be continued.

* For report of Subcommittee B and action by the Association, see *This Journal*, **28**, 56 (1945).

THURSDAY, OCTOBER 26

REPORT ON FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station, Burlington, Vt.), *Referee*

RECOMMENDATIONS*

It is recommended—

(1) That the method given in *This Journal*, **27**, 89 (1944) on "Sampling Feeding Stuffs in Bags," be made official, first action, and work continued:

It is also recommended—

(2) That line 2, 9, page 354, of *Methods of Analysis*, 1940, be deleted. This refers to the protein factor 5.7 for wheat grain.

(3) That the method for "Fat in Dried Milk Products" (modified Roese-Gottlieb Method), **24**, page 357, be made official, final action.

(4) That the method for "Water-soluble Acidity" **38**, page 363, be made official, first action.

(5) That methods for "Rice Hulls in Rice Bran," "Oat Hulls in Oats and Oat Feed," **44** and **45**, pages 364 and 365, be made official, first action.

(6) That the methods for "Grit in Poultry and Similar Feeds," "Bone in Meat Scraps or Tankage," "Calcium Oxide in Mineral Feeds" **46**, **47**, and **48**, page 365, be made official, final action.

(7) That the methods for "Ferrous Sulfate," "Copper Sulfate," and "Potassium Iodide" **52**, **53**, and **54**, page 367, be made official, final action.

(8) That the method for "Soluble Chlorine in Feeding Stuffs," *This Journal*, **26**, p. 87, with the following addition to first paragraph under "Procedure" **26**, page 88, after words "high in chlorine (over 2%)": "For mineral and other feeds containing over 10% chlorine weigh a one gram sample and use a 15 ml aliquot (representing one-tenth of total)" be made official, final action.

(9) That work on mineral feeds, lactose in mixed feeds, fat in fish meal, fat in cooked animal feeds containing cereals, crude fat or ether extract, activity of yeast, microscopic examination, fluorine, and mineral constituents in mixed feed be continued.

(10) That work on filtration aids in crude fiber determination, soluble chlorine and feeding stuffs, and ammoniacal urea and nitrogen salts be discontinued.

(11) That an associate referee be appointed to develop methods for evaluating the comparative quality of protein in fish and animal products.

(12) That an associate referee be appointed to study the crude fiber determination.

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 43 (1945). The methods, and other changes, will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

No reports were given by Associate Referees on sampling, mineral mixed feeds (calcium and iodine), lactose in mixed feeds, fat in fish meal, adulteration of condensed milk products.

REPORT ON FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS (ACID HYDROLYSIS)*

By STACY B. RANDLE (Kentucky Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

The Associate Referee did not present a written report on this subject last year, but an oral report was made upon the progress of the project. No collaborative investigation has been made since the last published report (*This Journal*, 26, 340). In this previous report, although there were only four collaborators, it was shown that generally the results obtained by the analysts were in closer agreement by the Bailey-Walker method than by the acid hydrolysis method. Also, the Associate Referee pointed out that acid hydrolysis of the residue after direct ether extraction yielded additional ether extract, and that the strength of the acid could be varied greatly without affecting the amount of ether extract significantly. In view of these findings, it was thought that the problem should be investigated further before undertaking more collaborative study.

As previously reported, emulsions are frequently encountered in the acid hydrolysis method. However, it is unpredictable which samples will emulsify the least; in fact, emulsion formation is also affected by the type and amount of shaking. When the alcohol was inadvertently omitted from a sample, it was found that the emulsion in this case was about the same as with the use of alcohol. However, the amount of ether extract was considerably less when the alcohol was omitted. Further investigation has shown that the alcohol may prevent emulsion and that it also increases the amount of ether extract. The same condition prevails when water is substituted for the alcohol. Since alcohol is not primarily a fat solvent, it is inconceivable that the addition of alcohol to the mixture could cause the extraction of more true fat than would the ether mixture itself. Table 1 shows that alcohol increases the amount of ether extract obtained from dog feeds in the acid hydrolysis method. For comparison, results obtained by the Bailey-Walker method are included.

In some cases in the acid hydrolysis method, the amount of ether extract obtained without the alcohol treatment agreed favorably with the results obtained by the Bailey-Walker method (note samples numbered 3600 and 3701). In other cases there was no correlation. The low results obtained without alcohol were evidently not due to the emulsion suspend-

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

ing the fat and holding it back, because in the sample numbered 3600 there was very little emulsion and in that numbered 3701 there was considerable emulsion formed. It is possible that the results obtained by the acid hydrolysis method were higher than those obtained by the Bailey-Walker method primarily because of the action of alcohol in extracting non-fatty alcohol-soluble materials which are not removed by the direct extraction with anhydrous ether. Further investigation is being made along this line.

Another approach is also being made to determine the type of materials extracted by the two methods, especially to determine the amount of glycerol in the extracts. The extracts obtained by the use of each method

TABLE 1.—*Effect of alcohol upon ether extract of acid hydrolysis mixtures—per cent ether extract*

LAB. NO.	BAILEY-WALKER	ACID HYDROLYSIS	
		WITH ALCOHOL	WITHOUT ALCOHOL
3599	4.63	5.58	3.50
3600	7.90	9.88	7.97
3601	5.48	6.31	3.07
3698	2.36	5.59	4.28
3700	1.70	4.51	2.79
3701	5.15	6.43	4.93

have been saponified separately, the fatty acids removed, and the glycerol oxidized to formaldehyde and formic acid. In the literature, there are several reports on determination of these oxidation products. One of the most recent of these is the polarigraphic method of Boyd and Bambach[1] for the determination of formaldehyde in biological materials. Arrangements were made with the Chemistry Department of the University of Kentucky to use the polarigraph in that department to study this method. However, unfortunately, the individual cooperating in this study left the University before satisfactory results were obtained. It is hoped this method can be studied later.

Schall and Thornton[2] have described a method whereby they were able to determine the true fat in extracts obtained by the Bailey-Walker method and the acid hydrolysis method. Their method necessitates the determination of the free fatty acids, the average molecular weight of the total fatty acids, and the glycerol content of the extracts. In this method the glycerol is oxidized with periodate and the oxidation products are determined volumetrically. Unfortunately, some of their procedures are described very meagerly. Workers in this laboratory have not found

[1] *Ind. Eng. Chem., Anal. Ed.*, 15, 314.
[2] *This Journal*, 26, 404 (1943).

sufficient time to duplicate all the procedures and corroborate the findings described in their paper. It is hoped that their method can be investigated thoroughly in the near future.

It is recommended* that this project be continued another year.

REPORT ON CRUDE FAT OR ETHER EXTRACT

By J. J. TAYLOR (State Chemist Florida Dept. of Agriculture, Tallahassee, Fla.), *Associate Referee*

This is a brief report on the work we have done in this laboratory on the two methods for determining fat in feed mixtures containing urea, using the official method of extraction, with ethyl ether, as compared with extraction with petroleum ether.

No attempt was made in this laboratory to get any collaborative work done on these samples, but several sets were run by each method merely as preliminary to recommending collaborative work for next year.

We are convinced, from the results of this work that the official method of extraction with ethyl ether gives erroneous results where feeds contain urea, and that much more concordant results are obtained by using petroleum ether as the solvent.

Two laboratory samples were prepared, one containing 9 percent of added urea and the other containing 3 percent. A 31 percent protein feed was selected to which was added 9 percent urea for Sample No. 1, and an 18 percent protein feed was selected for Sample No. 2, to which was added 3 percent urea.

Analysis—Sample No. 1, 31% Protein Feed, with 9% added Urea:

	percent
Protein	53.32
Protein (after fat extraction with ethyl ether dried in vacuum for 5 hrs.)	47.06
Protein (after fat extraction with ethyl ether without moisture removed)	36.11
Protein (after fat extraction with petroleum ether, without moisture removed)	50.96
Protein (on residue in fat extraction with ethyl ether)	11.85
Fat (dried in vacuum oven for 5 hrs., ethyl ether solvent)	5.95
Fat (dried in vacuum oven for 5 hrs., petroleum solvent)	4.30
Fat (without moisture removed, ethyl ether solvent)	10.35
Fat (without moisture removed, petroleum ether solvent)	4.35

It was noted in every case where ethyl ether solvent was used that large amounts of crystals of urea formed in the container upon drying out in the oven.

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 43 (1945).

It is of interest to note that there is only 0.05 percent of difference between the results obtained by using petroleum ether solvent when the sample was dried for 5 hours and when no moisture was removed; while in the case of the ethyl ether solvent almost twice the amount of extract was obtained when no moisture was removed from the sample before extraction as when it was heated for 5 hours before extraction; but even when the sample was dried for 5 hours the result was more than 1.50 percent higher than the results when petroleum ether was used as a solvent.

Sample No. 2, 18% Protein Feed, with 3% added Urea:	*per cent*
Protein	27.55
Protein (after fat extraction with ethyl ether, dried in vacuum)	24.70
Protein (after fat extraction with petroleum ether, dried in vacuum)	25.80
Protein (after fat extraction with ethyl ether without drying)	23.79
Protein (after fat extraction with petroleum ether without drying)	26.94
Fat (dried in vacuum oven 5 hrs., extracted with ethyl ether)	4.45
Fat (dried in vacuum oven 5 hrs., extracted with petroleum ether),	3.95
Fat (without removing moisture, ethyl ether solvent)	5.35
Fat (without removing moisture, petroleum ether solvent)	3.70

Again we see that there is greater loss of protein after extraction with ethyl ether, both after drying and without drying than there is when using petroleum ether; also, that with or without drying, the amount of fat or ether extract is greater where ethyl ether is used as a solvent than with petroleum ether, indicating that some urea is being dissolved by the ethyl ether.

As would be expected, the higher the percentage of urea in the sample the larger the difference between the ethyl ether and petroleum ether extracts.

It is recommended* that this subject be taken up as a matter for collaborative study next year.

REPORT ON FILTRATION AIDS IN CRUDE FIBER DETERMINATION

By L. S. WALKER (Agricultural Experiment Station, Burlington, Vt.), *Referee*

The Associate Referee has written to ten chemists from Maine to Texas with reference to crude fiber determination, and this report represents their combined experience, with that of the writer, on this problem.

One of the great difficulties in the crude fiber determination as outlined in our official method seems to be that of slow filtration of cottonseed meals and possibly other high-protein feeds. This slow filtration occurs when the final residue is transferred to the crucible, before drying.

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 43 (1945).

The concensus of the opinion of the ten chemists appears to be that very little trouble has been experienced in slow filtering, if certain precautions were followed. The addition of about 1 gram of asbestos, treated with acid and alkali, then thoroughly washed, burned and added before the last boiling, spreads the residue so that good filtration takes place in the porcelain Gooch crucibles used. The porcelain Gooch crucible with an asbestos pad proves to be superior to other types. The writer believes that, with proper manipulation, no trouble will appear and excellent checks can be obtained.

For the past three years, chemists from the trade and control services have been testing samples sent out under the direction of the Association of American Feed Control Officials. A study of the crude fiber results indicates wide variations in results—much wider variations than should exist. Individual laboratories obtain fairly concordant results, but, when all results are taken as a whole, they vary from one to six percent. The only explanation of this wide variation is that directions are not followed. Crude fiber is an arbitrary determination and its method should be carried out to the letter.

The method for crude fiber determination is one of the oldest in our book but not yet out of date; all chemists should study it from time to time, following carefully each part of the procedure.

REPORT ON SOLUBLE CHLORINE IN FEEDING STUFFS

By JOHN W. KUZMESKI (Massachusetts Agricultural Experiment Station, Amherst, Mass.), *Associate Referee*

No report will be made this year on "Soluble Chlorine in Feeding Stuffs," but the following recommendations are made in regard to the method published in *This Journal*, **26**, 87 (1943).

It is recommended*—

(a) That this sentence be added to the first paragraph under "Procedure," **26**, page 88, after the words "high in chlorine (over 2%)": "For mineral and other feeds containing over 10% chlorine, weigh one gram sample and use 15 ml. aliquot (representing one-tenth of total)."

(b) That the method as corrected be adopted as official, final action.

(c) That study of the method be discontinued.

No reports were given by the Associate Referees on ammoniacal urea and nitrogen salts, activity of yeast, and microscopic examination.

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* For report of Subcommittee A and action taken by the Association, see *This Journal*, **28**, 43 (1945).

REPORT ON FLUORINE IN FEEDS

By D. M. Doty (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

At the 1943 meeting of the Association it was recommended "that an associate referee on fluorine in feeds be appointed and that in the study of methods consideration be given to utilizing present procedures for fluorine in other substances as far as practicable." During the past year, therefore, the Associate Referee has attempted to review completely the literature on methods for the determination of fluorine and to make a decision with respect to the applicability of the various methods to the determination of fluorine in feeds.

Fortunately, extensive work has been carried out on methods for the determination of fluorine in soils, insecticides and fungicides, waters, brine, and salt, and foods. Careful consideration of the work done on these products led to the conclusion that a satisfactory method for feeds would involve the following: (1) Alkaline fixation and ashing, (2) distillation from perchloric acid in the presence of water (or steam), and (3) titration of the fluosilicic acid with thorium nitrate.

Very recently in our laboratories, Godfrey and Shrewsbury[1] have developed a method for the determination of fluorine in which these three steps are incorporated. Essentially the method consists of the ashing procedure recommended by Crutchfield,[2] the distillation procedure described by Willard and Winter,[3] and the titration technique recommended by Dahle et al.[4] The modifications suggested by Godfrey and Shrewsbury are, for the most part, refinements in apparatus and technique which are helpful in obtaining reproducible results. The method has worked so well in our laboratories (see results presented by Godfrey and Shrewsbury) and it is recommended* that it be studied collaboratively during the coming year. Any further recommendation must, of course, depend upon the results of this collaborative study.

No report on mineral constituents of mixed feeds was given by the Associate Referee.

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[1] Paper presented before the 1944 annual meeting of the Association of Agricultural Chemists, held at Washington, D. C. Oct. 25 and 26, 1944; published in *This Journal*, **28**, 335 (1945).
[2] *Ind. Eng. Chem., Anal. Ed.*, **14**, 57 (1942).
[3] *Ibid.*, **5**, 7 (1933).
[4] *This Journal*, **21**, 468 (1938).
* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 43 (1945).

REPORT ON FERTILIZERS

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

On account of the extra burden of war work, little collaborative work was done by the Associate Referees this year. The Associate Referee on Potash, and the Referee, have recommended several changes, which, while minor in character, should add to the accuracy of this method. The Referee recommends them for final adoption as official, with suspension of the by-laws.

The Associate Referee on Phosphoric acid has been working on the method for available phosphoric acid and for moisture, but has no recommendation for changes in these methods at the present time.

The Associate Referee on Acid-forming and Non-acid Forming Quality has recommended a change in the temperature of ignition used in the method for the purpose of securing greater accuracy.

The recommendation of the Associate Referee on Calcium is not approved. The previous recommendation was for further study, and no further study has been reported. We already have a volumetric method for calcium. Possibly an additional method for calcium may not be needed, or the gravimetric method may be made an optional part of the volumetric method. Further consideration is recommended.

The methods for water-soluble and acid-soluble boron were adopted in 1921, after damages to some crops were claimed to have been caused by boron in potash salts. Boron is now recognized to be an essential element and considerable work has been done on its determination in recent years. The methods for boron in fertilizers should be studied to see if any changes are needed to bring them up to date.

R. A. Osborn[1] states that addition of tartaric acid to the molybdate solution used in the volumetric acid for phosphoric acid prevents the ageing of the solution with consequent high results. The matter of ageing of this solution should be studied. It may be desirable to limit the time which the molybdate solution can be stored after the extra nitric acid has been added, or else to use tartaric acid when the solution is to be preserved for some time.

Defluorinated rock phosphate, made by fusing rock phosphate with silica, is now a commercial fertilizer. Fused tricalcium phosphate, calcium metaphosphate, and potassium metaphosphate may become commercial products. Methods for the determination of available phosphoric acid in these fertilizers should be studied.

The method for available phosphoric acid as it now reads applies to phosphates which did not exist at the time the method was adopted. A recommendation for correction was adopted as official, first action, in *This*

[1] *This Journal*, 25, 411 (1942).

Journal, 25, page 49, par. 27; the Referee has recommended the adoption of either one of two corrections which can accomplish the same purpose.

Defluorinated superphosphate is manufactured by treating rock phosphate with sulphuric acid and calcining the mixture. In some processes, both pyro and meta phosphates are formed. Experimental evidence indicates that the phosphate in such products are not utilized by animals as well as orthophosphates. Methods for the determination of soluble orthophosphoric acid and soluble metaphosphoric acid have been proposed by C. A. Butt, chief chemist, International Minerals and Chemical Company, East Point, Georgia, as follows:—

METHOD FOR SOLUBLE P_2O_5 AS ORTHOPHOSPHATES IN MINERALS FOR FEED

Weigh 0.4 gram sample (40 mesh) into 600 ml beaker. Add 400 ml of 0.4% HCl solution and stir for 2 hours at 98–99° F. Pour into 500 ml volumetric flask and make to mark with water. Shake to mix, and filter. Immediately pipette 100 ml (equiv. to .08 gram) into each of two 250 ml beakers. Add immediately 30 ml ammonium nitrate soln (40% soln.), 40–45 ml ammonium molybdate soln, and stir one of beakers for 20 minutes, and the other for 40 minutes, at room temp. (not over 30°C). Filter off and wash yellow precipitate immediately as each beaker is removed from stirrer. Titrate for percentage P_2O_5 in usual way. Ml of .3238 *N* NaOH required × 1.25 = % P_2O_5.

Deduct the result obtained by 20 minute stirring period from result representing the 40 minute period (to obtain percentage P_2O_5 representing the amount of orthophosphate formed by hydrolysis under the conditions in 20 minutes), and subtract this result from the result for the 20 minute stirring period to obtain the percentage P_2O_5 in the sample as soluble orthophosphate.

METHOD FOR SOLUBLE METAPHOSPHATE IN DEFLUORINATED PHOSPHATE

Weigh 0.4 gram of sample into 250 ml beaker. Add 200 ml 0.4% HCl solution and stir for 6 hours at 98–99°F. Make up to 250 ml volume, mix, and filter on dry filter. Pipette 12.5 ml into a 125 Erlenmeyer flask and neutralize with dilute ammonia (12 ml. water, 1 ml concentrated ammonia), using litmus paper. Dilute to 50 ml with water, add 10 ml of 5% acetic acid, then 10 ml of albumin solution (0.5 gram egg albumin in 100 ml of 5% acetic acid) and compare turbidity with standard.

Standard: Weigh 0.1 gram $NaPO_3$ (C.F.) into beaker and stir in 200 ml of 0.4% HCl solution until dissolved. Make up to 250 ml with water.

To a 225 ml Erlenmeyer flask add 10 ml 0.4% HCl solution, add 2½ ml water, and neutralize with dilute ammonia as above. Add 25 ml water, 10 ml 5% acetic acid, then 10 ml of albumin solution. To this solution add 0.25 ml of the $NaPO_3$ solution at a time until the turbidity shown by the sample is matched. 1 ml of the metaphosphate solution is equivalent to .0002784 gram P_2O_5 as metaphosphate.

The volume of the standard and aliquot of the sample must be kept at same volume during turbidity comparisons. Use Nessler tubes for final turbidity comparisons.

Tests of these methods by Mr. Butt are given in Table 1. As he states, results on Table 1 show that the methods are adequate, since the results are entirely in line with expectations. The insoluble P_2O_5 shown is presumed to be the insoluble form of metaphosphate. Analysis of a sample of commercial defluorinated superphosphate gave 29.08 per cent of total

P_2O_5 and 15.62 per cent of water-soluble ortho P_2O_5. These methods should be studied.

TABLE 1.—*Analysis products from mono-calcium phosphate, C. P., and di-calcium phosphate, C. P. after heating them for 30 minutes at 1000°C.*

	MONO-CALCIUM PRODUCT	DI-CALCIUM PRODUCT
	per cent	*per cent*
Total P_2O_5	71.79	55.65
P_2O_5 Insoluble in 0.4% HCl	55.97	0.00
P_2O_5 as Orthophsophate	0.00	0.62
P_2O_5 as Pyrophosphate	3.37	55.02
P_2O_5 as Metaphosphate, Soluble	11.85	0.01

RECOMMENDATIONS*

(1) That in the tentative method for "Acid-forming or base-forming quality," on p. 38, par. **60**, line 11, *Methods of Analysis*, 1940, the temperature of ignition be changed from 500–600°, to 575–600°. Tentative, final action.

(2) That to reagents for potash on p. 30, par. **40**, *Methods of Analysis*, 1940, a separate paragraph be added: "Acid alcohol. Mix 200 ml of 80 per cent alcohol with 20 ml of HCl and cool to room temperature." Editorial change. Final action as official.

(3) That to the determination of potash in **42(a)** p. 31, *Methods of Analysis*, 1940, the following changes be made: After the words, "Mixed fertilizers" and at the beginning of the first sentence add—"In a quartz, silica, or platinum dish of about 100 ml. capacity." After the first sentence add "The H_2SO_4 may be added after evaporation to dryness and before ignition." Change sentence, line 8 to read "Treat residue with about 6 ml of 80 per cent acid alcohol," and add the sentence "The temperature of the wash solutions should not exceed 30°C." Line 11, **42(a)** after the words "continue washing after filtrate is colorless," add the sentence "75 ml. is usually sufficient." (Final action as official with suspension of by-laws.)

(4) That the method for platinum recovery included in the report of the Associate Referee be considered for adoption as tentative after it has been published in the *Journal* as required by the by-laws (*This Journal*, vol. **25**, 1942, p. 125), and members of the Association have had opportunity to consider it.

(5) That on page 23, **12(b)** Book of Methods. "Not applicable in the presence of sulphates" be changed to "Not applicable to superphosphate and other fertilizers which contain sulphate." Editorial correction, final action as official.

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 44 (1945). The suggested changes were approved and will be incorporated in the revision of *Methods of Analysis*, *A.O.A.C.*, 6th edition, 1945.

(6) That either 17 or 16(b), page 24, be changed as given below. The change in 16(b) has already been adopted as official, first action (*This Journal*, **25**, page 49, par. **27** and page 314, par. **2**. The change in 17 accomplishes the same purpose but may be more desirable, since the interpretation of the analysis needs correction instead of the method. One change or the other should be adopted as official, final action.

That in paragraph 17, page 24, the last sentence be changed to read as follows: Subtract citrate-insoluble P_2O_5 from the total to obtain chemically available P_2O_5 in dicalcium phosphate, precipitated bone phosphate and precipitated bone. Or that paragraph **16(b)**, page 24, be changed to read as follows: "Dicalcium phosphate. Place one g of sample in 9 cm filter paper. Without previous washing, proceed as directed under **(a)** and determine P_2O_5 as directed under 9 or 12," Final action as official, either par. **17** or par. **16(b)**.

(7) That a study be made of methods for the determination of available phosphoric acid in fused tricalcium phosphate, calcium metaphosphate and potassium metaphosphate.

(8) That a study be made of methods for the determination of orthophosphoric acid, meta and pyro phosphoric acid.

(9) That a study be made of the ageing of the mohydrate solution used in the volumetric method for phosphoric acid to see if a time limit should be put on its use or an addition made to preserve it.

(10) That a study be made of the methods for water-soluble and acid-soluble boron to see if any changes should be made.

(11) That the work on potash and other subjects be continued as recommended by the Associate Referees.

(12) That Method I, official, for the determinaton of acid-soluble magnesium (52, p. 35) be deleted, but that such portions as are referred to in Method II (53, p. 36) be incorporated in Method II when revised. First action *This Journal*, **27** (1944) page 47, par. **18** (official, final action).

No reports were made by the Associate Referees on phosphoric acid (moisture) or on nitrogen.

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS

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

During the past year the applicability of the Neubauer soil-culture technic for the determination of the activity of various magnesium carriers

has been studied in this laboratory. Previous studies by the usual pot-culture methods have not been entirely successful, although soils known to be deficient in the field were used; this is probably because manipulation and aeration during preparation of the soils has liberated sufficient magnesium to decrease the deficiency, and to reduce the subsequent response of several test crops to magnesium fertilization. The Neubauer method provides for dilution of smaller quantities of soil with sand, cropping intensively with a relatively large number of rye or wheat seedlings for a short period, and the determination of magnesium removal from the different carriers relative to that from an active carrier such as magnesium sulfate. The effect of time, soil *p*H, and other factors, on decomposition of natural carbonates and processed oxides may be studied by mixing the carriers with the soils for varying time periods before cropping. It is hoped that the technic may help to establish values for the different carriers that will form a basis for further work with laboratory methods for active magnesium. The results that have been secured are promising but the number of trials is insufficient to justify presentation in this report.

No work with analytical methods was undertaken during the year, but the recommendations that follow are necessary to prepare for the new edition of the *Methods of Analysis*, 1945.

RECOMMENDATIONS*

It is recommended—

(1) That deletion of Method I, for the determination of acid-soluble magnesium, *Methods of Analysis*, 1940, **52**, 35, approved for first action last year (*This Journal*, **27**, 47), be given final action, but that the portions noted in the previous report (*This Journal*, **25**, 326, 1943) be incorporated in Method II when revised.

(2) That methods for magnesium and manganese be further studied.

REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS†

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

No collaborative work was conducted this year. The Associate Referee is of the opinion that no more work should be done for the present on the method for elimination of basicity due to material coarser than 20-mesh. Very good collaborative results were obtained on this method in 1943.[1] The probable limited use of this method may not justify its adoption as a tentative method.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 44 (1945).

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

[1] *This Journal*, **27**, 171 (1944).

CORRECTION FACTOR FOR ACIDITY DUE TO NITROGEN

Some consideration should be given to the correction for acidity due to nitrogen. The factor now being used is based on the theoretical and experimental data of Pierre[2] that one-half of the nitrogen is acid-forming. Pierre presented data from greenhouse experiments to show changes in hydrogen ion concentration and in exchangeable hydrogen of soils due to use of various nitrogen fertilizers. The relative values from his exchangeable hydrogen study were as follows: Ammonium sulfate, 100; ammonium phosphate, 100 to 104; urea, 42 to 50; ammonium nitrate, 42 to 55; calcium nitrate, −39; sodium nitrate, −42; calcium cyanamid, −55. Pierre also presented from experimental data the proportions of mixtures of various nitrogen fertilizers required to maintain a soil at its original reaction. It appears that data of this kind might be used to determine a practical value for calculation of acidity due to nitrogen. The writers have calculated the basicity or acidity of some of these mixtures from the titration and correction values of Pierre[3] and the results are given in Table 1. The titration value for ammonium nitrate is from this laboratory. The

TABLE 1.—*Application of the method to neutral mixtures of nitrogen fertilizers** (*Results in pounds $CaCO_3$ per ton*)

MIXTURE		ASH BASICITY	CORRECTION DUE TO N	NET
	per cent			
Sodium nitrate,	70			
Ammonium sulfate,	30	369B	635A	266A
Sodium nitrate,	54			
Urea,	46	623B	1078A	455A
Sodium nitrate,	54			
Ammonium nitrate,	46	662B	892A	230A
Cynamid,	65			
Ammonium sulfate,	35	796B	774A	22B
Cynamid,	52			
Urea,	48	1047B	1206A	159A

* To maintain the soil at its original reaction, as determined by Pierre.[3]

data show that 4 of the 5 mixtures give acid results varying from 159 pounds to 455 pounds calcium carbonate equivalent per ton of fertilizer, instead of the theoretical neutral value. The cyanamid-ammonium sulfate mixture was practically neutral. These results show the difficulty of ob-

[2] *J. Am. Soc. Agron.*, **20**, 254 (1928).
[3] *Ind. Eng. Chem., Anal. Ed.*, **5**, 229 (1933).

taining a practical correction value. They indicate, if the proportions of the various mixtures of nitrogen fertilizers are correct, that the present correction value is too large in most cases, possibly in all cases except where cyanamid is used. On the other hand, the results show the need of a correction factor, for without it all of the mixtures would appear to be strongly basic. Additional data on mixtures of nitrogen fertilizers which maintain a soil at its original reaction would be valuable.

COMPARISON OF ASHING TEMPERATURES OF 500 AND 600° C.

This was studied collaboratively last year and the results indicated that a temperature of 500°C. is too low to volatilize all the nitrogen. Some additional work on this subject was continued in this laboratory, using 2 molar solutions of sodium carbonate containing 25 and 50 grams of sucrose per liter respectively. The samples were 1-gram portions each consisting of 0.9 gram of a mixed fertilizer and 0.1 gram of dolomite. Ashing was made in an electric muffle equipped with a Wheelco Capacitrol temperature regulator. The nitrogen contents of the ashed samples are given in Table 2. The results show that not all the nitrogen is volatilized at 500°C. While the corrections would be small in most cases, there is a large

TABLE 2.—*Comparison of ashing temperatures of 500 and 600°C.*
(Results in per cent nitrogen)

SAMPLE* NUMBER	500° C		600° C	
	50 GRAMS SUCROSE	25 GRAMS SUCROSE	50 GRAMS SUCROSE	25 GRAMS SUCROSE
6423	0.21	0.18	0.04	0.01
6425	.03	.04	.03	.01
6519	.08	.04	.03	.01
6551	.13	.06	.03	.01
6605	.13	.07	.03	.01
6631	.47	.22	.07	.03

* 1 gram portions consist of 0.9 gram of the respective samples and 0.1 gram dolomite. The samples contained 6.07, 4.58, 3.87, 3.53, 2.08, and 6.08 per cent nitrogen, respectively. The respective percentages of water-insoluble nitrogen were 0.37, 0.44, 0.46, 0.44, 0.18, and 1.04.

amount of unignited carbon in samples ashed at 500°C., and in filtering, it is difficult to wash well and limit the volume of the filtrate to 100 ml. The smaller amount of sucrose in the sodium carbonate solution was more effective in volatilization of the nitrogen.

IGNITION IN THE PRESENCE OF NITRATE NITROGEN

Because of the increased amounts of ammonium nitrate used in mixed fertilizers, some attention was given to the evaporation and ignition procedures of the method when larger amounts of nitrate nitrogen are present. Using carbon black in place of sucrose with the sodium carbonate solu-

tion, no difficulty was experienced in the evaporation and ignition steps when ammonium nitrate in the sample was the equivalent of 4 per cent nitrogen in a mixed fertilizer and when the equivalent of 200 pounds per ton of organic material was present. When the sample consisted of 0.5 gram of ammonium nitrate or a mixture of ammonium and sodium nitrate an explosion was likely to occur in the furnace between 350 and 400°C., even when carbon black was substituted for sucrose. A 0.25-gram sample could be ignited without difficulty.

SUMMARY

Results of examining the accuracy of the present correction factor for acidity due to nitrogen, by applying the method to mixtures of nitrogen fertilizers which should give a neutral reaction, indicated that the present correction factor is too large in most cases. In comparisons of ashing temperatures of 500° and 600°C., the 500° temperature was too low to volatilize all the nitrogen. A sample can be evaporated and ignited successfully with the larger amounts of nitrate nitrogen present in mixed fertilizers if carbon black is substituted for sucrose.

RECOMMENDATIONS

It is recommended*—

(1) That in Ch. II, par. **60**, p. 38, line 11, the figures 500–600° be changed to 575–600°.

REPORT ON POTASH AND PLATINUM RECOVERY METHODS†

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

In accordance with the recommendations of the Association "that work on the details of the method for the determination of potash be continued, including the method for recovery of platinum" (*This Journal*, **27**, 45–47, 1944), referee work was conducted this year by collaboration. A copy of the proposed work was sent to each of thirty-eight chemists who had expressed a willingness to collaborate. This report summarizes the results of the fourteen who found time to do the work and make a report of it to the Associate Referee.

Collaborative work on potash in fertilizers for 1944 was directed to 4 subjects:

(1) Recovery and purification of platinum from the determination of potash in fertilizers.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 44 (1945).

† Journal Paper Number 193 of the Purdue University Agricultural Experiment Station.

(2) Comparison of effects of 80% acid-alcohol and 80% alcohol with those of 90% acid-alcohol and 90% alcohol.

(3) Determination of the effects of temperature on the solubility of potassium chloroplatinate in acid-alcohol and alcohol.

(4) Effects of adding sulfuric acid at the beginning or near the end of the evaporation.

COMMENTS ON RECOVERY OF PLATINUM

For the past four years a platinum recovery method has been sent to the collaborators asking them to try it out, and comment on it, so that, in a revised form, it could be offered to the Association for adoption. This year the method has met with enough favorable comment to warrant its further revision and preparation for submission to the Association. The Associate Referee, in submitting the method, is well aware that many chemists have their own platinum recovery and purification method and that perhaps it will not be used by many of them even if it is adopted; nevertheless, it is time that a method should be made available to those who have a need for one.

(1) RECOVERY AND PURIFICATION OF PLATINUM FROM THE DETERMINATION OF POTASH IN FERTILIZERS

I. RECOVERY OF PLATINUM

(A) RECOVERY OF PLATINUM FROM THE ALCOHOL WASHINGS

(1) Allow the ammonium chloride washings to run into the flask with the alcohol washings. Allow the ammonium platinum chloride to settle, decant off the supernatant liquid, and save the platinum salt. Reduce as in B 1 or B 2.

(2) Evaporate the alcohol waste in a porcelain dish on a steam bath or an electric hot plate. (A piece of filter paper in the dish prevents most of the platinum from sticking to the dish.) Filter on a Büchner funnel and wash the reduced platinum. Transfer to a porcelain dish and ignite at about 700°C. in a muffle for about 20 minutes. Digest the reduced platinum in a porcelain dish on a steam bath with several portions of (1–3) hydrochloric acid. Repeat until the solution is colorless. Wash well with distilled water until a test with silver nitrate shows that all chlorides have been removed. Digest with a few portions of (1–4) nitric acid, wash, dry, and weigh.

(3) Acidify the alcohol waste with hydrochloric acid. Add either 20-mesh zinc, or aluminum in stick or sheet form (for volumes of 75 to 150 ml. of acid use 10 to 20 grams of metal) and allow to stand until all the platinum is reduced. Filter, ignite at 700°C. as in (2).

(B) RECOVERY OF PLATINUM FROM THE K_2PtCl_6 SALT

(1) Dissolve the K_2PtCl_6 in 20 parts or more of hot water, acidify with hydrochloric acid and reduce with either 20-mesh zinc, or aluminum in sheet or stick form. Filter and ignite as in A 2.

(2) Dissolve the K_2PtCl_6 in water and precipitate as ammonium chloroplatinate with ammonium chloride. Allow to stand several hours, filter on a Büchner with suction, and wash with alcohol. Transfer to a porcelain dish and ash in a muffle, first at a low temperature (about 200°C.) for about 20 minutes and finally for 30 minutes at high heat (about 700°C.).

(3) Dissolve the K_2PtCl_6 in 20 parts or more of boiling water. Add sodium formate slowly, a pinch at a time, stirring well at each addition (excessive foaming may occur with a resultant loss of platinum unless great care is exercised). Complete reduction* is indicated by the solution becoming colorless. Filter and ignite as in A 2.

II. PREPARATION OF PLATINUM SOLUTION

Dissolve the platinum from I in a porcelain dish on a steam bath with three parts of conc. HCl and one part conc. HNO_3. After solution evaporate with additions of conc. HCl for three times, to remove the excess of HNO_3, and then with distilled water for three times, to remove the excess of HCl. Do not evaporate below ½ the original volume. Filter and make to calculated volume. Evaporate and test a 10 cc portion, or one equivalent to one gram of platinum, for material insoluble in 80% alcohol. In case a blank shows the presence of impurities the platinum in the solution should be reduced, purified, and made up again. To determine the strength of the solution evaporate 2 ml in a porcelain dish with an excess of potassium sulphate of about 0.5 gm. Add alcohol and wash the K_2PtCl_6 as in the determination for potash. The solution may be made up so that 1 ml equals 1% K_2O in a 1 gm sample.

(2) COMPARISON OF 80% ACID-ALCOHOL† AND 80% ALCOHOL, WITH 90% ACID-ALCOHOL† AND 90% ALCOHOL

I. SUGGESTED PROCEDURE

Prepare sufficient solution of sample x according to **41**(a), p. 31, to make 6 determinations on each treatment, using 80% and 90% alcohols as referred to above

Approximate K_2O value of sample $x = 12$.

(3) DETERMINATION OF THE EFFECT OF TEMPERATURE ON THE SOLUBILITY OF K_2PtCl_6 IN ACID-ALCOHOL AND ALCOHOL

I. SUGGESTED PROCEDURE

a. Prepare a sufficient solution of samples according to **41** (a), p. 31, to make 12 potash determinations by **42** (a), p. 31, with the following modifications:

1. Determine the potash in the first 6 samples by cooling to about 25°C. both the acid-alcohol and the 80% alcohol used as wash in the determinations.

2. Determine the potash in the second 6 samples by warming to 35°C. both the acid-alcohol and the 80% alcohol.

3. In each case, use 75 ml. of alcohol for the wash preceding the NH_4Cl wash, and 50 ml. of 80% alcohol for the one following it, and break up the potash salt with a policeman. Keep the acid-alcohol mixture, while in contact with the potash salt for the 15-minute period, at the proper specified temperature (either 25° or 35° C.).

(4) EFFECT OF ADDING H_2SO_4 AT THE BEGINNING OR NEAR THE END OF THE EVAPORATION

I. SUGGESTED PROCEDURE

a. Prepare sufficient solution of Sample x to make 12 potash determinations.

1. Determine the potash in the first 6 samples as directed in **42** (a), p. 31, evaporating the aliquot to dryness before adding the H_2SO_4 and burning off.

2. Determine the potash in the second 6 samples as directed in **42** (a), p. 31, but adding the H_2SO_4 before the aliquot has been evaporated to dryness.

To chemists reporting collaborative potash results under (2), (3) and (4) the questionnaire outlined below was sent; a summary of their replies follows.

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* Complete reduction may be tested as follows: Pipette about 25 ml. of the clear solution into a 250 ml. beaker, add a few drops of hydrochloric acid and small amount of potassium iodide solution. If platinum is not completely reduced, the solution will turn red unless a trace of nitric acid is present.

† Acid-alcohol mixture should be cooled or allowed to cool before being used.

1. Type of filter . . . (a) If glass sinter list porosity number (b) asbestos padded Gooch, (c) special padded Gooch, or (d) . . . ?
2. Volume of alcohol used for washing (ml).
3. Temperature of alcohol used for washing (°C.).
4. Type of dish used for ignition: Pyrex, porcelain, platinum, or . . . ?
5. Capacity of dish used for ignition (ml).
6. Size of aliquot used for determination (¼ or ½ gram).
7. Evidence of visible insoluble residue.
8. State if results were obtained by direct weighing, . . . or by dissolving out the K_2PtCl_6 and weighing back. . . .
9. List the individual results as well as the average.

RECOMMENDATIONS*

It is recommended—

(1) That the condensed and revised method for recovery and purification of platinum be adopted by the A.O.A.C. (Official first action).

(2) That an upper limit of 30°C. be accepted as a safe working temperature for the acid-alcohol and alcohol used in washing the K_2PtCl_6 precipitate (or) that all acid-alcohol and alcohol be saturated with K_2PtCl_6 at room temperature before using. (Official, first action.)

(3) That collaborative work on potash in fertilizers by the present A.O.A.C. method using concentrations of acid-alcohol and alcohol 80% and above be continued.

(4) That Par. 42, page 31, lines 1 to 3 be expanded and changed to read: "In about 100 ml. dish, add to a 25 or 50 ml. aliquot of solution **(41a),** to which has been added sufficient potash-free normal NaOH (1–2 ml.) to prevent formation of free phosphoric acid during ignition; add 1 ml. H_2SO_4 (1+1), etc.; or, evaporate the above prepared aliquot to dryness in about 100 ml. dish and then add 1 ml. H_2SO_4 (1+1)," etc. (Official, first action).

(5) That Par. 42, page 31, lines 8 to 13 be expanded and changed to read as follows: "Treat the residue with about 6 ml. of 80% acid-alcohol (0.6 ml. concentrate HCl to 6 ml. 80% alcohol cooled or allowed to come to room temperature before using) breaking up the residue with a policeman. After 15 minutes at 30°C. or less, either transfer the residue direct to the crucible (Gooch or sinter) using for this operation about 75 ml. of 30°C. or less of 80% alcohol. Wash 5 or 6 times with 10 ml. portions of NH_4Cl solution to remove impurities, then wash 5 times with 10 ml. portions of 30°C. or less of 80% alcohol, and dry precipitate 30 minutes at 100°C." etc. (Official, first action.)

(6) That collaborative work on potash in fertilizers be done on filtration equipment with a thought to comparing such filtration means as glass sinters with specified porosities, asbestos padded Gooches, asbestos paper padded Gooches, etc.

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 44 (1945). The method as revised will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

(7) That collaborative work on potash in fertilizers be done with reference to the possibility of improving the present official method by substituting magnesium chloride for sodium hydroxide. (It is thought by some that more accurate and higher results will be obtained in this way).

(8) That some collaborative work on potash in fertilizers be done with reference to the addition of materials to the solution to speed up the filtration and clarification of the final solution. (Special reference is cited of one worker using ½ g. of Dicalcite on solutions of fertilizers containing a large amount of organic matter.)

(9) That some collaborative work on potash in fertilizers be done with reference to the uniformity of results obtained by the use of various sized dishes with special reference to the size of the aliquot that can be safely used for a definite sized dish.

(10) That some collaborative work on potash in fertilizers be done with reference to the uniformity of results obtained by the use of varying volumes of acid-alcohol and alcohol. (For this work I would suggest that both the acid-alcohol and alcohol used for the washes be saturated with K_2PtCl_6 at room temperature.)

COMMENTS OF COLLABORATORS

(1). 80% alcohol unsafe in hot weather, a few degrees rise in temperature can easily run alcohol down to 78 or 79%. Personally I favor a minimum of 85% alcohol; many other factors are more the cause of poor results than even 95% alcohol would involve. H_2SO_4 added at the beginning of evaporation is not only a time-saving operation, but causes less spattering when burning the sample off.

(2). I believe some collaborative work should be done to provide for the use of magnesium chloride and 95% alcohol.

(3). No platinum recovery method should be specified, as many are now in use and one is about as good as another.

(4). Although it is not necessary for the A.O.A.C. to have a method for the recovery of platinum I would favor the adoption of a method for use by the Association.

(5). I would favor the adoption of a method of recovery of platinum by the Association. I have found that direct weighing is unsafe, since it may frequently yield results out of line, to the extent of as much as .15%. In some cases, there was visible residue, but in most cases there was none, even in cases in which weighings demonstrated that residues were present.

(6). I favor correction for insoluble residue, as gains in crucible weights ranged from .0005 grams to .0103 grams—averaging .0041 grams. This weight on ¼ gram aliquot is equivalent to 0.32% K_2O. In the direct weight method, this weight gain is considered potash and is calculated as such. This is erroneous and such results are always too high.

(7). No comment.

(8). Our usual practice in potash determinations is to use 95% alcohol and 80% acid-alcohol. Results by direct weighing—no evidence of visible residue.

(9). When 90% alcohol was used, some visible residue was noticeable. I prefer to use 80% alcohol previously saturated with K_2PtCl_6. In all determinations, a small insoluble residue was noticeable—indicating that direct weighing would give re-

sults that are too high—results reported were obtained by dissolving out the K_2PtCl_6 and weighing back.

(10). Method for platinum recovery is desirable, at any rate, if not necessary. Noted insoluble residue—results reported obtained by dissolving out the K_2PtCl_6 and weighing back.

(11). Results obtained by direct weighing. Although there was a slight amount of visible insoluble residue. Increase of temperature of alcohol to 35° apparently dissolves some of the potash salt.

(12). Favor adoption of a platinum recovery method by the Association.

(13). The A.O.A.C. should provide a method for the recovery of platinum for those who do not have one worked out satisfactorily. Results were obtained by direct weighing. To overcome the difference in solubility of acid-alcohol and alcohol washes at various temperatures, I would suggest the solutions be saturated with K_2PtCl_6 as is done with the ammonium chloride wash.

(14). Results by direct weighing—no evidence of visible residue.

COMMENTS ON RESULTS OF TABLES 1, 2, 3, AND 4

The results of the chemists who collaborated on the potash work are reported in these tables. In Table 1, with one exception, all of the chemists

TABLE 1.—*Effect of concentration of acid-alcohol and alcohol on determination of K_2O in fertilizers.*

[Sample X (0–0–12)]

ANALYST NO.	ANALYSES MADE		METHOD A: 80% ACID-ALCOHOL AND ALCOHOL			METHOD B: 90% ACID-ALCOHOL AND ALCOHOL		
	NO.	°C	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE
1	6	25	12.88	12.77	12 81	13.10	13.04	13.07
2	6	25	12.70	12.55	12.65	12.90	12.75	12.85
3	6	25	13.01	12.87	12.96	13.03	12.88	12.95
4	6	30	12.54	12.47	12.50	12.64	12.56	12.61
5	6	25	12.98	12.90	12.94	13.05	12.90	12.99
6	6	25	12 72	12.40	12.49	12.96	12.44	12.66
7	6	25	12.92	12.60	12.71	13.56	13.04	13.22
8	6	25	12.88	12.78	12.82	12.90	12.84	12.88
9	6	25	12.64	12.54	12.59	12.87	12.74	12.79
10	6	25	12.88	12.70	12.79	12.99	12.85	12.89
11	11	30	13.15	12.90	13.01	13.25	13.00	13.12
12	6	28	12.62	12.53	12.57	12.74	12.69	12.72
13	6	R*	12.69	12.63	12.66	12.79	12.65	12.71
14	6	30	13.04	12.96	13.00	13.12	12.96	13.05
Average at		25	12.85	12.68	12.75	13.04	12.83	12.92
Maximum variation at		25	0.37	0.50	0.47	0.92	0.60	0.61

* R = Room temperature.

reporting obtained higher values with the use of the stronger alcohol. The over-all average difference between the determinations showed a gain of

0.17% potassium oxide in favor of the use of the stronger alcohol for those doing the work at 25°C. This difference has been shown to vary with fertilizers of varying potash contents and with alcohols of varying concentrations.[1,2] Since these differences can be obtained in any laboratory under

TABLE 2.—*Effect of temperature of acid-alcohol and alcohol on determination of K_2O in fertilizers*
[Sample X(0–0–12)]

ANALYST NO.	ANALYSES MADE	METHOD A: WASHED WITH ALCOHOL AT 25 °C.			METHOD B: WASHED WITH ALCOHOL AT 35°C.		
		HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE
1	6	12.88	12.77	12.81	12.76	12.70	12.73
2	6	12.70	12.55	12.65	12.40	11.80	12.05
3	6	13.01	12.85	12.92	12.86	12.63	12.70
4	6	12.62	12.55	12.59	12.53	12.41	12.47
5	6	12.95	12.88	12.92	13.01	12.90	12.96
6	6	12.68	12.55	12.62	12.85	12.29	12.61
7							
8	6	13.08	12.82	12.91	12.94	12.82	12.88
9	6	12.64	12 54	12.59	12.65	12.57	12.60
10							
11	11	13.09	12.88	12.93	12.67	12.35	12.52
12	6	12.70	12.64	12.65	12.65	12.60	12.62
13	6	12.69	12.61	12.64	12.67	12.45	12.59
14	5	13.06	12.92	13.00	13.12	12.90	13.02
Average		12.84	12.71	12.77	12.76	12.53	12.65
Maximum variation		0.47	0.38	0.38	0.72	1.10	0.97

controlled conditions, this work should be continued another year, but with more explicit conditions set forth by the Associate Referee in directions to the collaborators, so that the differences reported in the results will be in even better agreement than those reported in this work.

In Table 2, whereas one should expect to obtain lower potassium oxide values at the higher temperature, three of the twelve that reported obtained about the same or slightly higher values at the higher temperature. This can only mean that their laboratory conditions were not well controlled. The over-all average of nine out of twelve that reported showed a loss of 0.12% potassium oxide when the alcohols were heated to 35°C. over that when heated at 25°C. Part of the nonuniformity of results, reported in this table, can be assigned to the varying types of laboratory equipment and the varying laboratory conditions under which the work was done. The varying conditions reported in Table 4 of this report are directly

[1] Allen, H. R., *This Journal*, 22, 162–167 (1939).
[2] Hughes, C. W., and Ford, C. W., *Ind. Eng. Chem., Anal. Ed.*, 14, 217 (1942).

responsible for the irregularities found in the analyses of the first three tables.

As a result of two years collaborative study of the effect of temperature of alcohols on the solubility of potassium chloroplatinate, in addition to work by Hughes and Ford,[3] the Associate Referee is convinced that some definite upper temperature limit should be established for the washing alcohols used in the determination of potash in fertilizers; and this is recommended to the Association for approval.

In Table 3 the results obtained in the comparison agreed remarkably well with work obtained in past years. This is not surprising, as many chemists have reported that repeated comparisons of this in their own

TABLE 3.—*Effect of time of addition of H_2SO_4 on determination of K_2O in fertilizers*
[Sample X(0–0–12)]

ANALYST NO.	ANALYSES MADE		METHOD A: H_2SO_4 ADDED AT START OF EVAPORATION			METHOD B: H_2SO_4 ADDED NEAR END OF EVAPORATION		
	NO.	°C.	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE
1	6	25	12.86	12.76	12.83	12.88	12.77	12.81
2*	6	25	13.10	13.00	13.04	13.10	13.00	13.05
3	6	25	13.01	12.85	12.92	13.05	12.87	12.96
4								
5	6	25	12.86	12.98	12.93	13.00	12.90	12.95
6	6	25				12.72	12.40	12.49
7								
8	6	25	13.00	12.86	12.93	13.00	12.84	12.91
9	6	25	12.64	12.54	12.59	12.63	12.59	12.62
10								
11	11	30	13.05	12.95	12.99	13.05	12.95	12.98
12								
13	6	?	12.69	12.20	12.57	12.75	12.20	12.53
14	6	30	13.00	12.88	12.92	13.04	12.92	12.95
Average			12.91	12.78	12.86	12.92	12.74	12.83
Maximum variation			0.46	0.80	0.47	0.47	0.80	0.52

* 95% alcohol used instead of 80%.

laboratories show that there is no measurable difference in the potassium oxide values obtained by either procedure. The Associate Referee believes that either procedure is safe and that the method should be worded so that either procedure could be used. The addition of the sulfuric acid at the start of the evaporation has been found by those who have tried it out to be a time saver, and since time saving was one of the objectives for this part of the work, it could well be incorporated into the method without

[3] Hughes, C. W., and Ford, C. W., *Ind. Chem. Chem., Anal. Ed.*, 13, 233–234 (1941).

TABLE 4.—*The reported equipment with which the 1944 collaborative potash work was done*

COLLABORATORS	TYPE OF FILTER	VOLUME ALCOHOL USED	TEMP. OF ALCOHOL °C	TYPE DISH	CAPACITY DISH	SIZE ALIQUOT	EVIDENCE OF VISIBLE RESIDUE	RESULTS BY DIRECT OR INDIRECT WEIGHT
		ml			*ml.*	*gram*		
1	asbestos padded Gooch	150	25	platinum	75	0.1 or 0.2	none	indirect
2	all sinter and asbestos	75	28	silica	80	¼	none	direct
3	asbestos Gooch	150	?	silica	85	0.3876	(?)	direct
4	sinter M. poro.	100 & 125	25	platinum	75	¼	trace	direct
5	pyrex sinter M	125	25	platinum	100 & 75	¼	trace	indirect
6	asbestos padded Gooch	125	25	porcelain	60	¼	yes	indirect
7	asbestos padded Gooch	125	Room ?	porcelain	125	¼	none	indirect
8	sinter M.	120	23–26	platinum	100	¼	none	direct
9	pyrex sinter M	125	25	platinum	80	¼	yes	indirect
10	pyrex sinter M and asbes.	80	26	platinum	75	¼	trace	indirect
11	sinter M	120	30	silica	60	0.2425	trace	direct
12	asbestos padded Gooch	125	28	platinum	100	¼	none	direct
13	asbestos padded Gooch	125	room?	platinum	125	¼	none	direct
14	Gooch paper asbestos	125	30	platinum	100	¼	none	(?)

affecting the accuracy of the potash results; and this part of the work could then be discontinued.

Table 4 lists the conditions and the equipment with which the collaborative potash work was done in 1944; and a study of these should help to explain the irregularities reported. Most chemists realize that the following factors affect the accuracy of the results they report:

The type of filter if slow or fast; the volume of alcohol used, whether small or large; the temperature of the alcohols; type of dish and the volume of the dish whether large or small for the size of the aliquot to be used; presence of visible residue; and whether or not the determination is to be made by direct or indirect weighing.

A study of collaborative results by the Associate Referee for the past four years, from the standpoint of the type of equipment used in making the results reported, has led him to believe that more uniformity with reference to these items mentioned in Table 4 would do much to enhance the value of the potash work. Fortunately, part of this standardization can be incorporated in the changes in the method, but improvement will have to come about principally through the chemists themselves. It is hoped that a study of Table 4, in connection with results reported in Tables 1, 2, and 3, will help to bring more uniform laboratory conditions, and thus more consistent results.

LIST OF COLLABORATORS

(1) W. R. Austin, Armour Fertilizer Works, Nashville, Tenn.
(2) C. R. Byers, Armour Fertilizer Works, Carteret, N. J.
(3) R. D. Caldwell, Armour Fertilizer Works (Factory), Atlanta, Ga.
(4) F. D. McSwiney, Wilson & Toomer Fertilizer Co., Jacksonville, Fla.
(5) H. C. Batton, Swift & Company Fertilizer Works, Baltimore, Md.
(6) E. F. Boyce, Chemist in Charge Regulatory Service, Vermont Agr. Experiment Station, Burlington, Vt.
(7) R. Earle Dickey and H. H. Hanson, State Board of Agriculture, Dover, Del.
(8) Katherine W. Ford and Mary C. Fox, Davison Chemical Corp., Baltimore, Md.
(9) C. Tyson Smith and Philip H. Smith, Massachusetts State College, Agricultural Experiment Station, Amherst, Mass.
(10) M. W. Goodwin, Southern States Laboratories, Baltimore, Md.
(11) T. L. Ogier and G. S. Fraps, Texas A. and M., College Station, Texas.
(12) W. Chapman, Consolidated Rendering Co., Boston, Mass.
(13) R. M. Smith, Agricultural Department, Chemical Division, Tallahasee, Fla.
(14) H. L. Moxon and R. O. Powell, Virginia-Carolina Chemical Corp., Richmond, Va.

No reports were given on calcium and sulfur, or copper and zinc, by the Associate Referees.

REPORT ON PLANTS

By E. J. MILLER (Agricultural Experiment Station, East Lansing, Mich.), *Referee*

The following reports from Associate Referees were received:

(1) Report on Carotene in Plant Tissue, by Erwin J. Benne, Dorothy I. Rose, A. Joyce Satchell, and Elva L. Denniston.

(2) Report on Copper in Plants, by Lillian I. Butler and E. J. Benne.

(3) Report on a Method for the Determination of Iodine in Plant Material, by J. S. McHargue.

(4) Report on Iron in Plants, by Erwin J. Benne and A. Joyce Satchell.

RECOMMENDATIONS*

On the basis of the above reports and the results of work reported during the past 5 years, the following recommendations appear to be substantiated:

(1) That the procedures for the determination of carotene in plant tissue described in the report on that subject, together with the supplementary information given, be included in the Plant chapter of the revised *Methods of Analysis*.

(2) That cross references be given in the Plant chapter to all the other procedures for the determination of carotene.

(3) That the study of methods for the determination of carotene in plant tissue be continued and that as many as possible of the promising procedures for this purpose be investigated.

(4) That the method for copper now given in Sections 20 and 21 of the Plant chapter be deleted.

(5) That the method given in the report on that subject be accepted as a tentative method in place of the one deleted.

(6) That work be continued on methods for the determination of this constituent of plant material.

(7) That Section 40 of the Plant chapter be amended to include the changes suggested by Dr. McHargue.

(8) That the colorimetric thiocyanate method for iron determination given in the present *Methods of Analysis* be deleted.

(9) That the colorimetric o-phenanthroline method as given in the report on the subject be accepted as an official method, first action, to replace the thiocyanate method.

(10) That the titanous chloride titrimetric method be accepted as an official method, first action.

(11) That work on methods for determining iron in plant ash be con-

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 45 (1945). Details of the methods will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

tinued and as many as possible of the promising procedures for this purpose be investigated.

(12) That Method 28 in the Plant chapter be reworded for the 6th edition of *Methods of Analysis*, 1945.

No report was given on sampling by the Associate Referee.

REPORT ON IODINE AND BORON IN PLANTS

By J. S. McHARGUE (Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

A revised method for the determination of iodine in plant material is recommended for tentative adoption, in place of the present method (*Methods of Analysis*, **XXII**, 40).*

A colorimeter method for determination of boron in plant material was studied in conjunction with boron in soils.† A procedure for total boron in plants is recommended for tentative adoption. It is also recommended that Methods I, **28**, **29**, and **30**, for boron in soils be deleted.

No reports were given by Associate Referees on carbohydrates or zinc in plants.

REPORT ON COPPER IN PLANTS

By LILLIAN I. BUTLER, *Associate Referee* (Bureau of Human Nutrition and Home Economics, Beltsville, Md.), and E. J. BENNE (Chemical Section, Michigan State College, East Lansing, Mich.)

Work on the determination of copper in plant materials has been continued, and the method found satisfactory by the Associate Referee is proposed for acceptance. Except for minor changes, this method is the same as that published by Butler and Allen in the Report on Copper and Cobalt in Plants for 1941.[1]

RECOMMENDATIONS‡

It is recommended—

(1) That the method for copper now given in Chapter **XII**, sections **20** and **21** be deleted from *Methods of Analysis*.

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* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 45 (1940).

† See report on Boron and Fluorine in Soils, pp. 797. Both of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] *This Journal*, **25**, 467 (1942).

‡ For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 45 (1945). Details of the proposed method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

(2) That the method given herewith be accepted as a tentative method in place of the one deleted.

(3) That work be continued on methods for the determination of this constituent of plant material.

REPORT ON CAROTENE IN PLANTS

By ERWIN J. BENNE, *Associate Referee*, and DOROTHY I. ROSE, A. JOYCE SATCHELL, and ELVA L. DENNISTON (Agricultural Experiment Station, East Lansing, Mich.)

Interest in the analysis of plant tissue for its carotene content continues, and a number of adaptations or modifications in methods for this purpose have been published since the reports on carotene were prepared in 1940 and 1941 (1). To investigators familiar with this field it is obvious that the determination of carotene in plant material involves three distinct operations: *viz.*, I. Extracting the pigment from the tissue, II. Isolating it from other substances in the extract, and III. Evaluating the amount in the final solution. Recently, the greatest emphasis has been upon II, and the principle of chromatography has been used intensively by numerous investigators. In connection with these chromatographic investigations studies have been made of the spectral characteristics of certain carotinoids commonly associated with beta carotene in plant material, with the hope of making possible the quantitative evaluation of each constituent in a mixture of these substances. Taking, preparing, and preserving samples of plant material for the analysis of carotene still present perplexing problems. A number of the more recent contributions to the literature on this general subject are given in the brief review which follows.

REVIEW OF LITERATURE

Beadle and Zscheile (2, 3) have presented quantitative absorption data which make possible the spectrophotometric analysis of a mixture of beta and neo-beta carotene for the content of the individual pigments. These authors confirmed the presence of the latter pigment in fresh plant material and concluded that it was a product of the isomerization of beta carotene, and the same substance formerly designated as pseudo-alpha carotene. Schrenk, Silker, and King (4) made use of this method to study the kinetics of the isomerization of beta carotene. They concluded that such isomerization is responsible for the variation in correction values reported for use in the spectrophotometric analysis of vitamin A in a mixture of carotinoids. These authors (5) also studied the effects of such isomerization on analytical values for carotene in extracts of dehydrated alfalfa and suggested a procedure which they believe holds interference from this source to a minimum. Mann (6) in a study of the chromato-

graphic separation of plant pigments found that alumina would adsorb neo-beta carotene more strongly than beta carotene. He believes that the former pigment is a congener of the latter but does not agree that it is the same as pseudo-alpha carotene. Kemmerer and Fraps (7) and Kemmerer, Fudge, and Fraps (8) have reported upon the carotene content of a considerable number of plant materials and have given values not only for two neo-beta-carotenes but for several other isomers as well.

White and Zscheile (9) made a quantitative study of the distribution of beta-carotene, crytoxanthol, and zeaxanthol between hexane and various aqueous solutions of methanol, diacetone alcohol, and 2-methyl-2, 4-pentanediol. Values for the water content of the hypophasic solvent best suited for the separation of these carotinoids were given. White, Zscheile, and Brunson (10) studied the carotinoids of yellow corn grain and gave preliminary quantitative absorption spectra of neocryptoxanthol and two neozeaxanthol isomers. These authors (11) have also presented a method for the spectrophotometric evaluation of the carotinoid constituents of this grain.

Haskin (12) reported a method for the determination of the carotenes and other major leaf pigments. The pigments were extracted with hot methanol followed by chromatographic separation and spectrophotometric evaluation.

Bolton and Common (13) developed a modification of Moon's method for the determination of carotene in silage which included chromatographic isolation of carotene by use of a column of dicalcium phosphate.

Kemmerer (14) reported on the details of a collaborative study of methods for the determination of what was designated as crude and pure carotene by complete and abridged chromatographic methods. The author concluded that if the amounts of different carotene isomers, which closely resemble each other physically and chemically but differ in vitamin A potency, are to be determined the complete chromatographic method is necessary.

Curtis (15) described a method for determining carotene in leaf tissue which made use of a chromatographic column of starch.

Wall and Kelly (16) reported the development and use of a chromatographic method for the determination of carotene. Results from this method, as compared with those from the Peterson-Hughes-Freeman technic, indicated that considerable amounts of non-carotene pigments are estimated by procedures making use of the methanol phasic separation of xanthophyll from carotene. These authors feel that their procedure is sufficiently rapid to be well adapted to routine analysis.

Haagen-Smit, Jeffreys, and Kirchner (17) described a procedure for the determination of carotene in which xanthophylls and carotene were separated by use of ortho-phosphoric acid.

Charkey and Wilgus (18) have presented a chromatographic method

for the determination of carotene which they believe avoids oxidative losses of carotene as well as errors due to incomplete extraction and incomplete separation of carotenes from other pigments present. Their article includes an enzyme inactivation and sample storage procedure that is said to make possible the collection and storage of samples of fresh plant tissue for analysis at a later date.

RECOMMENDED METHODS

It is the opinion of the authors that the methods proposed are as well adapted for the routine determination of total carotene in plant tissue as any that are available at present. If it is desired to determine only pure beta carotene it is necessary to resort to more involved chromatographic procedures which as yet are less convenient for the analysis of large number of samples. Hence, on the basis of the information given in the 1940 and 1941 Reports (1) and the experience of the authors, it is recommended:

(1) That the procedure proposed be adopted as tentative methods by the *A.O.A.C.*, and that they, together with the supplementary information given, be included in the Plant chapter of the revised edition of *Methods of Analysis*.*

(2) That cross references be given in the Plant chapter to all information on the determination of carotene in *Methods of Analysis*.

(3) That the study of methods for the determination of carotene in plant tissue be continued and that as many as possible of the promising procedures for this purpose be investigated.

LITERATURE CITED

(1) *This Journal*, **24**, 526, 1941; **25**, 573, 1942.
(2) *J. Biol. Chem.*, **144**, 21, 1942.
(3) *Ind. Eng. Chem.*, **14**, 633, 1942.
(4) *Ibid.*, **16**, 328, 1944.
(5) *Ibid.*, **16**, 513, 1944.
(6) *Analyst*, **69**, 34, 1944.
(7) *Ind. Eng. Chem., Anal. Ed.*, **15**, 714. 1943.
(8) *J. Am. Soc. Agron.*, **36**, 683, 1944.
(9) *J. Am. Chem. Soc.*, **64**, 1440, 1942.
(10) *Ibid.*, **64**, 2603, 1942.
(11) *Ind. Eng. Chem., Anal Ed.*, **14**, 798, 1942.
(12) *J. Biol. Chem.*, **144**, 149, 1942.
(13) *J. Soc. Chem. Ind.*, **61**, 50, 1942.
(14) *This Journal*, **25**, 886, 1942.
(15) *Plant Physiol.*, **17**, 133, 1942.
(16) *Ind. Eng. Chem., Anal. Ed.*, **15**, 18, 1943.
(17) *Ibid.*, **15**, 179, 1943.
(18) *Ibid.*, **16**, 184, 1944.

* For report of Subcommittee A and action by the Association, see *This Journal*, **28**, 45 (1945). The details of the method will be published in the 6th edition of *Methods of Analysis, A. O. A. C.*, 1945.

REPORT ON IRON IN PLANTS

By ERWIN J. BENNE, *Associate Referee*, and A. JOYCE SATCHELL
(Agricultural Experiment Station, East Lansing, Mich.)

Work on methods for determining iron in plant ash has been continued since preparation of the 1941[1] and 1943[2] reports on this subject. During this time the colorimetric o-phenanthroline method for this purpose has continued to demonstrate its superiority over the colorimetric thiocyanate method in the 1940 edition of *Methods of Analysis*. The details of the former method found satisfactory by the authors will be published in the revision of *Methods of Analysis*, 1945.

On the basis of the results of the collaborative study given in the 1943 Report and the experience of the authors, it is recommended*—

(1) That the colorimetric thiocyanate method given in the present *Methods of Analysis* be deleted.

(2) That the colorimetric o-phenanthroline method as proposed by accepted as an official method, first action, to replace the thiocyanate method.

(3) That the titanous chloride titrimetric method be accepted as an official method, first action.

(4) That work on methods for determining iron in plant ash be continued and as many as possible of the promising procedures for this purpose be investigated.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

Because of absence of and depletion in personnel, the studies during the year have been restricted to the laboratories of the Associate Referees, W. M. Shaw and J. S. McHargue.

A preliminary report was made by the Associate Referee for Liming Materials. He has conducted an exhaustive study of the factors that affect potentiometric titrations to establish the neutralization value of blast furnace slag and the quenched calcium silicate slag from rock phosphate reduction furnaces. Values so obtained have been integrated with those obtained by the abbreviated procedures specifically appropriate for conversion of the charges of the two types of slag to combinations susceptible of direct titrative determinations of Calcium Carbonate-equivalence. Be-

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[1] *This Journal*, 25, 556, 1942.
[2] *Ibid*, 27, 526, 1934.
* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 45 (1945). Details of the method will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

cause of variance in the contents of alumina, and in the incidence of phosphates and fluorides, it was imperative to develop different analytical technics for the two types of slag. These technics are being subjected to further and collaborative study.

The Associate Referee for Less Abundant Elements (Boron and Fluorine) in Soils has reported studies on the determination of boron. Advantage was taken of the several researches that post-dated those upon which were based the procedures given in the present sections 28, 29, and 30 of the 1940 Edition of *Methods of Analysis*. Using these researches as the background for his own studies, the Associate Referee has recommended the deletion of the above-mentioned sections and the respective substitutions of the sections stipulated in his report for the determination of total and available boron.

In correspondence with interested personnel of governmental agencies, it has been pointed out that in the obtaining of samples of soil intended for both mechanical and chemical analyses, the material of minus 2 mm. is deemed appropriate for the reserve, or stock, sample. Cognizance is taken of this agreed specification for the bulk sample in the directions for sampling, with continued recognition of the fact that the charge and fineness of the fraction to be subjected to analysis will be stipulated for specific determinations.

It is recommended*—

The editorial clarifications and inclusions suggested in the general revisions for Chapters I and IV be approved.

No report on hydrogen-ion concentration of soils of arid and semi-arid regions was given by the Associate Referee.

REPORT ON BORON AND FLUORINE IN SOILS

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

The work this year was limited to boron. The colorimetric procedure by Berger and Truog[1] using quinalizarin appears to be the most satisfactory of any of the several published methods for the determination of boron in soils and plant material. The chief disadvantage of the method is that it requires the preparation, handling, and storage of 98 percent of

* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 45 (1945). The details of changes in the method will be published in the 6th edition of *Methods of Analysis*, A.O.A.C., 1945.

[1] Berger, K. C., and Truog, E., "Boron determinations in soils and plants using the quinalizarin reaction." *Ind. Eng. Chem. Anal. Ed.*, 11, 540–545 (1939).

sulfuric acid. This disadvantage is offset however by other important advantages, such as extreme sensitivity of the reagent for the determination of small amounts of boron and the rapidity with which results may be obtained after the extract of the soil or plant ash is obtained.

Studies by McHargue and Hodgkiss,[3] DeTurk and Olson[4] and by Berger and Truog[2] have resulted in improvements in the method as originally outlined by the latter authors. Particular improvements include the use of the photoelectric colorimeter for making the color comparisons. Any of the instruments that are in common use are satisfactory provided they are equipped with suitable filters of known spectral transmittance. Recent spectrophotometric studies of the boron-quinalizarin complex made in this laboratory comfirm the findings of other investigators that the strongest absorbtion takes place at a wave length of 600 millimicrons. Therefore, color comparisons should be made using a filter that transmits light of this wave length, since the highest sensitivity and greatest range of scale readings on the instrument are obtainable only when this is done. This study also confirmed the fact that the relation between the color and boron concentration does not conform precisely to Beer's Law, so that points on the standard curve must be determined at small intervals over the entire working range.

Several factors affect the slope of the curve and should be carefully noted. Small changes in concentration of sulfuric acid in the solution as prepared for comparison have a marked effect on the intensity of the color, the greatest sensitivity being obtained if the acid closely approaches a concentration of 98 percent by weight of sulfuric acid. The standard curve should thus be checked frequently to be certain that no changes have occurred in the reagents and, always, when a new lot of reagents are to be used.

Temperature also has an important effect on the color intensity, the sensitivity decreasing rapidly with increase in temperature.[2,3] Since a temperature of 25°C. is usually obtainable, it is suggested that all color comparisons be made at this temperature.

The tubes or cuvettes to be used to hold the solution when the comparisons are made should be tested for uniformity and those showing variation should be discarded or correction factors applied. Tubes or cuvettes made of Pyrex glass should not be used unless the readings are to be made immediately after the solution is transferred to them.

The procedures are essentially those described by Berger and Truog.

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[2] Berger, K. C., and Truog, E., "Boron tests and determination in soils and plants." *Soil Sci.*, 57, 25–36 (1944).

[3] McHargue, J. S., and Hodgkiss, W. S., "Report on boron and fluorine in soils." *This Journal*, 25, 311–313 (1942).

[4] Olson, L. C., and DeTurk, E. E., "Rapid microdetermination of boron by means of quinalizarin and a photoelectric colorimeter." *Soil Sci.*, 50, 257–264 (1940).

RECOMMENDATIONS*

The authors suggest that the procedure they have described be substituted for the method given in the 1940 edition of *Methods of Analysis, A.O.A.C.*, which is too laborious for the routine examination of large numbers of soils. While the suggested method for available boron is in our belief the best that we have at present, further study should be made of it as a means of detecting the extent of boron deficiency in the arable soils.

No reports were given by the Associate Referees on zinc and copper, exchangeable calcium and magnesium, and exchangeable hydrogen.

REPORT ON LIMING MATERIALS

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

No further work has been done during the past year upon either the determination of exchangeable calcium and magnesium, or upon exchangeable hydrogen in soils. The Associate Referee has given considerable time, however, to a study of the differential solubilities of various types of calcium silicate slags in carbonated water as affected by origin, crystallinity, fineness, and other variables. It was anticipated that the findings would be helpful in the development of analytical procedures for the determination of the neutralization value of silicate slags in their use as liming materials.[1]

The tentative procedure for slags recommended last year[2] is now being subjected to collaborative study of representative samples of blast furnace slags distributed by producers of this type of slag, and the results are being awaited.

It is recommended that the tentative procedure for the neutralization value of slags be continued until further collaborative results shall have been obtained.

APPOINTMENTS

R. E. Bergman, State Department of Agriculture, St. Paul, Minnesota, has been appointed Associate Referee on Sampling and Analysis of Condensed Buttermilk.

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* For report of Subcommittee A and action by the Association, see *This Journal*, 28, 45 (1945). The details of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] See paper entitled "Titrative Determination of the Neutralization Value of Calcium Silicate Slags" in the preceding number of *This Journal*, p. 310.

[2] *Ibid.*, 27, 532 (1944).

CONTRIBUTED PAPERS

INVESTIGATION OF THE IMMERSION REFRACTOMETER METHOD OF DETERMINING METHANOL AND ETHANOL IN MIXTURES OF THE TWO ALCOHOLS

By GEORGE F. BEYER and PAUL A. REEVES (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C., and Philadelphia, Pa., respectively)

The junior author had occasion to analyze a distillate containing a mixture of methanol and ethanol and decided to use the immersion refractometer method as outlined (p. 176) in *Methods of Analysis*, of the Associa-

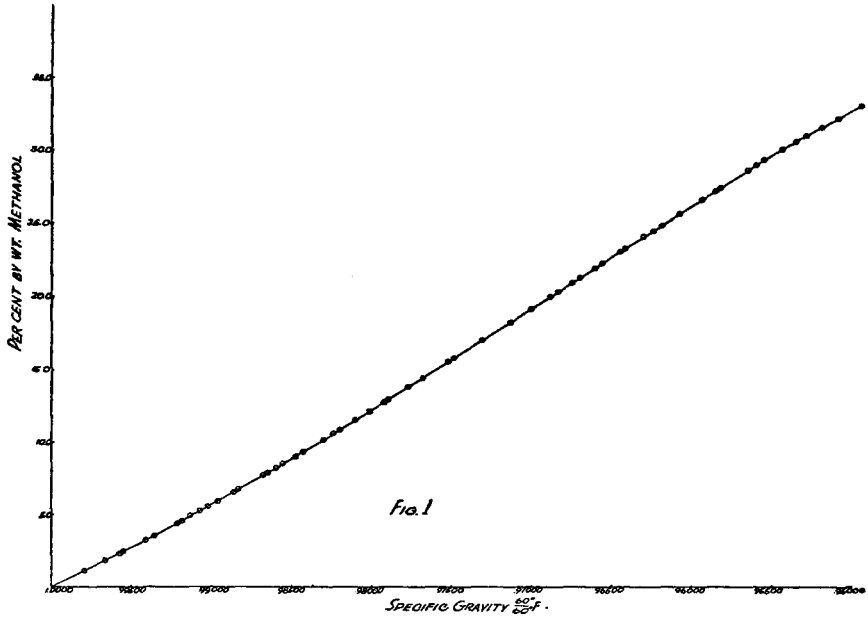

FIG. 1.—Specific gravity plotted against per cent by weight for methanol.

tion of Official Agricultural Chemists, 5th Edition. However, he noticed that the method of calculation there used merely gave the proportion of the alcohols present and not the per cent of each in the sample. Therefore, he set about to investigate this section of the chapter on distilled liquors.

In his approach to this problem, he prepared dilute solutions of each alcohol having, as nearly as possible, the same specific gravity, but not

the same per cent of alcohol. Mixtures of these solutions were made, refractometer readings were taken and the percentage of each alcohol was calculated from these data. An examination of his results and some of the data on which they were based showed that the scale readings he obtained for ethanol did not agree with those in reference table 20.[1] However the two scale readings for methanol that could be directly compared did

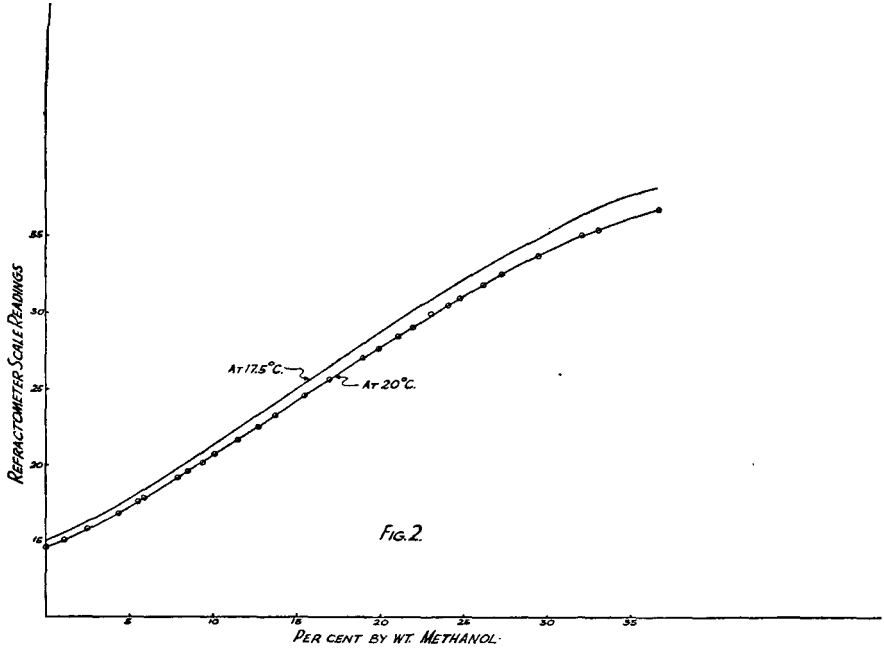

FIG. 2.—Refractometer readings against per cent by weight, methanol.

agree with those obtained in this investigation. At this point the Washington laboratory undertook the investigation of this problem in an effort to solve the difficulty.

EXPERIMENTAL

The methanol used for this purpose was purified by treating it with "Drierite" ($CaSO_4$) distilling through a 36″ column approximately 0.5″ in diameter, packed with half-turn pyrex glass helices. The design of the distilling head was such that the ratio of reflux to take-off could be controlled as desired—in this case it averaged about 3 to 1. The boiling point of the alcohol was 64.5°–64.7°C. at 765 mm. The ethanol used was re-

[1] *Methods of Analysis, A.O.A.C.*, 5th ed. (1940).

distilled in a similar still and its purity or strength was determined by taking the specific gravity at 15.56°/15.56°C. Then standards of various strengths by weight of each alcohol were prepared, their specific gravities were determined at 15.56°/15.56°C., and their respective Zeiss immersion refractometer readings were taken at 17.5°C. and 20.0°C. From these data curves were constructed by plotting specific gravity against per cent by weight (Fig. 1), and refractometer readings against per cent by weight (Fig. 2). Only the curves for methanol are shown in Fig. 2, since an

TABLE 1.—*Table showing specific gravity, scale readings at 17.5°C. and 20.0° C. and per cent by weight of methanol.*

METHANOL, % BY WT.	SP. GR. 60°/60° F.	SCALE READINGS AT 17.5°C.	SCALE READINGS AT 20°C.
1	0.99820	15.45	14.95
2	.99635	15.95	15.5
3	.99460	16.5	16.0
4	.99290	17.15	16.6
5	.99120	17.75	17.2
6	.98960	18.45	17.85
7	.98795	19.10	18.5
8	.98635	19.80	19.2
9	.98475	20.45	19.85
10	.98320	21.2	20.55
11	.98175	21.9	21.25
12	.98030	22.6	21.95
13	.97885	23.35	22.65
14	.97740	24.1	23.4
15	.97600	24.85	24.1
16	.97455	25.65	24.8
17	.97315	26.4	25.55
18	.97170	27.2	26.25
19	.97030	27.95	26.95
20	.96885	28.7	27.6
21	.96740	29.4	28.3
22	.96595	30.1	29.0
23	.96450	30.8	29.7
24	.96305	31.45	30.35
25	.96165	32.1	31.0
26	.96020	32.75	31.65
27	.95880	33.35	32.3
28	.95730	33.95	32.85
29	.95590	34.5	33.45
30	.95440	35.15	33.95
31	.95280	35.7	34.5
32	.95115	36.25	34.95
33	.94950	36.75	35.35
34	.94780	37.25	35.75
35	.94615	37.65	36.15
36	.94455	37.95	36.50

examination of the curves for ethanol showed that the scale readings agreed with those listed in reference table 20,[1] and in table 27, page 177.[1] The scale readings obtained for methanol at 17.5°C. also agreed with those in that table, except between the specific gravities of 0.9800 and 0.9760. An examination of the data collected so far shows that the scale

TABLE 2.—*Per cent of each alcohol in 17 samples*

COMPOSITION OF SAMPLE		CALCULATED ACCORDING TO PAGE 177, 5TH ED. A.O.A.C. USING DATA IN TABLE 27		CALCULATION ACCORDING TO REEVES USING SCALE READINGS AT 17.5°C.		CALCULATION ACCORDING TO REEVES USING SCALE READINGS AT 20.0°C.	
% METHANOL	% ETHANOL	% METHANOL	% ETHANOL	% METHANOL	% ETHANOL	% METHANOL	% ETHANOL
12.85	8.05	13.07	7.86	12.89	7.95	12.88	8.07
8.45	21.61	8.39	12.55	8.37	21.57	8.33	21.62
6.11	15.63	6.22	15.50	6.21	15.52	6.15	15.59
6.56	15.03	6.70	14.96	6.68	14.98	6.57	15.10
4.37	10.01	4.60	9.83	4.48	9.99	4.35	10.10
17.08	14.10	16.84	14.25	17.02	14.07	16.92	14.17
11.53	9.56	11.80	9.40	11.71	9.50	11.61	9.60
13.70	9.33	13.93	9.14	13.91	9.17	13.80	9.29
13.67	15.84	13.82	15.61	13.80	15.63	13.73	15.71
12.78	8.05	12.93	7.98	12.89	8.03	12.77	8.17
17.35	8.17	17.34	8.15	17.34	8.13	17.31	8.17
5.08	17.09	5.28	16.93	5.28	16.93	5.19	17.04
3.18	10.85	—	—	—	—	3.21	10.77
8.97	20.93	9.12	20.86	9.12	20.86	9.11	20.87
4.04	10.72	4.25	10.57	4.20	10.62	4.12	10.71
9.75	4.63	9.93	4.52	9.83	4.64	9.84	4.62
6.86	7.31	7.11	7.14	6.99	7.25	6.89	7.35

readings obtained for either alcohol do not agree with those published by Leach and Lythgoe.[2]

In view of the information thus obtained by experimentation, and of that obtained from the National Bureau of Standards regarding the accuracy of the Zeiss immersion refractometer readings for ethanol in reference table 20,[1] Table 1 was compiled from Figs. 1 and 2 showing specific gravity, and scale readings at 17.5°C. and 20.0°C., corresponding to per cent by weight of methanol.

The junior author's preliminary experiments, and those performed in this investigation, have conclusively shown that solutions of ethanol and methanol having the same specific gravity do not contain the same per cent of alcohol. The percentage composition of the sample must therefore be calculated according to his suggestion, which is to take into consideration the per cent of *each* alcohol corresponding to the specific gravity of the sample. In order to determine the accuracy of the data thus far obtained,

[1] *Loc. cit.*
[2] A. E. Leach and Hermann C. Lythgoe, *J. Am. Chem. Soc.*, **27**, 964 (1905).

17 samples containing varying amounts by weight of ethanol and methanol were prepared. From the specific gravity and the Zeiss immersion refractometer readings taken at 17.5°C. and 20.0°C. the percentages of the respective alcohols were calculated by 2 different methods and tabulated (Table 2). The percentages found in Columns 3 and 4, using data in Table 27, p. 177, were obtained by first calculating the proportion according to the A.O.A.C. method and finishing the calculation according to the above suggestion. The figures in the other columns, using scale readings at 17.5°C. and 20.0C°., respectively, were obtained according to this suggestion, which follows in detail:

Ascertain the per cent of ethyl alcohol by volume corresponding to the specific gravity of the sample at 15.56°/15.56°C., convert by use of Table 21[1] to per cent by weight and designate it as *E*. In like manner find from Table 1 the per cent of alcohol as methyl by weight and designate it as *M*. From reference Table 20[1] obtain the Zeiss immersion refractometer reading *A*, corresponding to the per cent of ethanol by volume at either 17.5°C. or 20.0°C., preferably 20.0°C. (The reason for preferring this temperature will be pointed out later.) In like manner obtain from Table 1 scale reading *B* (at the same temperature as reading *A*) corresponding to *M*. Take the immersion refractometer reading of the sample itself at 20.0°C. and designate it as *R*. Then, since the addition of methanol to ethanol decreases the refractive index in direct proportion to the quantity present, use the following formula in calculating the respective alcohols. $A-R/A-B=P$ the fractional portion of the sample to be calculated as a mixture of methanol and water of the same specific gravity as the sample. Then $1-P$ equals the fractional portion to be calculated as a mixture of ethanol and water of the same specific gravity.

Since *M* per cent of the sample is methanol if it were all methanol, then $P \times M$ equals per cent by wt. of methanol in the sample. By the same reasoning $(1-P) \times E$ equals per cent by wt. of ethanol in the sample.

Example:—Sp. gr. at 15.56°/15.56°C. = 0.96849 = 27.16% by vol. as ethanol or 22.27% (*E*) by wt. From Table 1 it corresponds to 20.2% (*M*) by wt. as methanol. Immersion scale reading of sample at 20.0°C. = 37.8 (*R*).

Immersion scale reading for 27.16% ethyl alcohol by vol. from reference Table 20,[1] equals 55.5. From Table 1 the scale reading for 20.2% by weight of methanol is 27.75. Then 55.5 − 27.75 = 27.75, the difference in the refractometer readings for these percentages of the respective alcohols. The difference between the refractometer reading of ethyl alcohol alone and that of the sample is 55.5 − 37.8 = 17.7. This divided by 27.75 = .6378, the fractional portion to be calculated as a mixture of water and methanol. Then 1.0000 − 0.6378 = 0.3622, the fractional portion to be calculated as a mixture of water and ethanol. Therefore, 0.6378 × 20.2% = 12.88%, the

per cent by wt. of methanol and $0.3622 \times 22.27\% = 8.07\%$, the per cent by wt. of ethanol in the sample.

Or, substituting in the formula $(A-R)/(A-B) \times M = (55.5-37.8)/(55.5-27.75) \times 20.2 = 12.88\%$ by wt. methanol; and $(1-P) \times E = 0.3622 \times 22.27\% = 8.07\%$ by wt. ethanol in the sample.

The last three samples listed in Table 2 were prepared and analyzed principally for the purpose of determining whether or not the scale readings (especially for methanol) in Table 27, page 177,[1] for solutions having specific gravities at 15.56°/15.56°C. between 0.9800 and 0.9760, are more nearly correct than those obtained in this investigation. In order to fulfill all the conditions deemed necessary for this purpose, the ratio of methanol to ethanol in these samples was 1 to 2.5, 2 to 1, and 1 to 1, respectively. An examination of these results shows, almost conclusively, that the Zeiss immersion refractometer readings for methanol, as listed in Table 27, page 177,[1] within the specific gravity range just mentioned is slightly in error, or at least outside the limits of legitimate experimental error. Attention is called to the results obtained at the two different temperatures (17.5°C. and 20.0°C.). A comparison shows that in the majority of cases those closest to the actual were obtained when the immersion refractometer readings taken at 20.0°C. were used in the calculations. It is for this reason that this temperature is given preference.

Attention is directed to Column 2 in Table 2. The figures in this column were obtained by the use of the suggested formula, but the data used in the formula were taken from Table 27, page 177.[1]

CONCLUSIONS

This investigation has clearly shown that mixtures of ethyl alcohol, methyl alcohol, and water can be quite accurately determined by the specific gravity at 15.56°/15.56°C., the Zeiss immersion refractometer reading at 20.0°C., by calculating the result as shown in the example. However, if the methanol content is less than about 2% in the sample prepared for the refractometer reading, this method is not recommended. The modified Deniges[3] method should be used instead. Attention is called to the fact that the specific gravity of the sample under examination must be brought within the range of Table 1, if it is not already there.

[3] *Methods of Analysis, A.O.A.C.*, 5th ed. (1940), p. 176, XVI, 23.

THE USE OF STATISTICS IN BIOLOGICAL EXPERIMENTATION AND ASSAY*

By LILA F. KNUDSEN (Food Division,† Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Clarity of design, of purpose, and of analysis should be the guiding light in all biological experiments and assays. The function of statistics is to furnish a lens to clarify the image, rather than to blur or obscure it with a maze of figures and formulas. T. Swan Harding has said with fine irony: (7) "Now obscurity has its uses, as the Lord God of Hosts discovered when he wanted to stop the children of men from building the Tower of Babel. He simply passed a miracle and got them each to speaking a different tongue, whereupon progress upward and onward ceased abruptly"; and again: "Even today some writers, like some preachers, deliberately phrase their material in difficult words or technical jargon in order to acquire prestige by shooting over the heads of their audiences."

Statistics in biological experimentation can be expressed simply and in plain, everyday English. The concepts, basic assumptions, and interpretations do not require technical jargon. The arithmetical procedures of calculations, and the various steps, variance tables, etc., in a statistical analysis usually need not be given, since they are merely the means of arriving at a conclusion, in the same way as are the details of a chemical test for arsenic to find out whether or not arsenic is present. It is only necessary, in either case, to give a reference to where that particular type of procedure or method can be found. J. Ansel Anderson has given an excellent talk on the simplicity and common sense of statistics. (2)

DESIGN OF EXPERIMENT

Statistics has a very important, though little known, use in biological (and chemical) work, namely in the planning of the experiment or assay. It is quite the usual practice to hold constant all but one cause of variation and to thus measure the effects of this one cause. Frequently, this procedure does not show what actually happens, since it assumes, among other things, that the causes of variation are independent of each other. The ordinary planning of an experiment cannot hope to see through the complicated maze of cause and effect sufficiently to separate the various causes and evaluate their interdependence. This is where statistical methods can be of great assistance. There are certain mathematically-hewn designs or plans that are so arranged and so balanced as to remedy some of the defects of the usual plan. Usually, the use of one of these statistical designs will attain the same precision as the ordinary design,

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25, 26, 1944.

† W. B. White, Chief.

with fewer animals and in a shorter time. It is thus advantageous whether or not statistical methods are used in the interpretation. A good source book is Fisher's "Design of Experiments" (4) or Fisher and Yates' tables (5).

As an illustration, suppose a comparison is desired between three diets and a control diet, and also an evaluation of the effects of sex and season of the year on these comparisons. Suppose also that there is a difference in response between the litters which are under observation and that no more than 3 males and 3 females (6 animals) can consistently be obtained from each litter. The following design (a "randomized incomplete block" design) might be repeated each season. Male and female are designated as M and F.

	Control diet	Diet 1	Diet 2	Diet 3
Litter 1	M&F	M&F	M&F	
Litter 2	M&F	M&F		M&F
Litter 3	M&F		M&F	M&F
Litter 4		M&F	M&F	M&F

Unless data are available on individual variations within the control group and within the experimental groups, it is advisable to have equal numbers of animals in each group. The order of setting up the experiment may influence results. Suppose, for example, one is comparing the effects of three diets on the growth of rats and is first selecting from a large cage of rats all the rats to be put on Diet 1. Naturally, the least active rats will be caught first and thus put on Diet 1. These rats will start the experiment under a handicap of being potentially less healthy, and therefore may grow more slowly than the rats caught later and put on the other diets. Thus the experiment has a definite bias before it is even started; and even though all the diets may be essentially equal, Diet 1 might well be shown "inferior" by such a faulty experiment. The difficulty could be obviated by putting each animal on each diet *in a random manner*. "Random manner" indicates some entirely objective, unbiased method of choosing the diet for each rat. The diets could for example be numbered, and these same numbers placed on metal-rimmed disks. These disks could be shaken in a bag and, as each rat is caught, one number could be chosen, sight unseen, to determine what his diet should be. Alternatively a die could be shaken, or one could use a table of random numbers such as is given by Tippet (12) or Fisher and Yates (5). Thus in each seasonal repetition there would be 6 rats on each diet.

In a table of designs the one shown above might be given as:

abc, abd, acd, bcd

where each group of three letters indicates a litter, and each letter a diet or control diet.

A design similar to the above was used in a clinical experiment to determine the relationship between the potency of penicillin and the pain produced at three different sites of injection. The potency was arbitrarily divided into 6 equal intervals. Each patient was given a dose of penicillin in each of three sites of injection. The design used was taken from Fisher and Yates, (5) Table XVII, and reads:

abc	bcf
abd	bde
ace	bef
adf	cde
aef	cdf

A different letter was assigned to each one of the six intervals of potency, in random order. Each of the 10 three-letter groups indicated a different patient. The above design was repeated three times so that 30 patients were used in the experiment. For the first 10 patients the injections were given in site 1 first, site 2 second, and site 3 third. For each of the other two sets of 10 patients, the order of the sites of injection was changed (*e.g.*, site 2 was given first, site 3, second, etc.).

Of course, in applying any of these statistical designs, use must be made of all previous background knowledge accumulated during numerous earlier experiments. Also there may be causes of variation that one does not suspect, such as the temperature of the laboratory, or an unsuspected inhomogeneity in the diet of the animals. There may be a seasonal, or even daily, change in the characteristic being measured, and so on. Many hitherto unsuspected sources of variation have been spotted through the use of a statistically designed experiment.

INTERPRETATION OF RESULTS

No measurement of any kind, whether dimensional, chemical, or biological, can be exact. This applies particularly to biological determinations. In the weighing of a rat, for instance, there will be variations in the weight of an adult rat from day to day, and even on the same day, since it will weigh less before it has been fed than afterward. This is an example of how, statistically, all variations can be divided into two types: (a) those due to chance and (b) those due to assignable causes. The principal problem of statistics is to distinguish between these two types of causes so that those reasonably attributable to the effects of the drug, food or chemical under investigation, will be disclosed. The idea of "tolerances" involves the first class of variations which cannot be cured but must rather be endured, and must therefore be measured as closely as possible. In all investigations it is important to know the size of these variations-due-to-chance. Such measurement involves, among other things, a pretty good picture of how much individual animals vary in the particular char-

acteristic being measured, even when they are as identical as human planning can make them. In comparing the averages of the characteristic measured, for control group and for experimental group, it is important to know how great a difference between these averages may be due to chance alone. Statistics furnishes the best tool for testing this so-called "significance of a difference." In biological assays it is also important to know what is the variation due to chance—in other words, the variation in a series of several "identical" assays. This is called the "error of the assay" and is analogous to the "error of analysis" of the chemist. This subject will be enlarged upon later.

Of course, statistics is sometimes grossly misused. Everyone has heard of the person who throws out all data that do not support his conclusions and applies "statistics" to the remaining results. A couple of illustrations of possible misuse follow:

One might be a complicated transformation involving the growth curve of a rat. The growth curve of an animal is, of course, the plotting of the weight each week against the age of the animal (or the number of the week). A complicated transformation could be used for the purpose of making a straight line out of the growth curve. What the users may overlook, however, is that instead of the influence of each of the 52 weekly weights of the rat being equal, the "straight line," by such a device, is determined almost entirely by the first six or seven weights of the rat, statistically ignoring the remaining 45 weights.

Another misuse of statistics could occur in comparing two different biological methods for determining the same thing. Obviously, in order for the two methods to be comparable enough for one to be substituted for another, the methods must have the same precision and the same accuracy, that is, they must give the same average results with the same amount of error. If one method gives an average of 30 ± 2, the other method would not be comparable if it gave an average of 45 ± 2 on the same substances; though the errors of the methods are equal, the methods themselves obviously do not measure the same thing. In a comparison of two such methods, statistics might be used to confuse the issue with many manipulations and formulas, so that the equal errors of the methods appear to be all important and the fact that one method gives consistently higher average results appears to matter not at all.

STATISTICS AS APPLIED TO BIOLOGICAL ASSAY

There are two main types of biological assay, from a statistical viewpoint: (1) those giving a measurable quantitative response, *e.g.*, gain in weight of rat in a vitamin A assay, per cent of ash of tibia in a vitamin D assay, diameter of zone of inhibition in a penicillin assay, amount of digitalis used to kill a cat; and (2) those giving a "go or no go" type of response wherein an individual animal either survives or dies, or an individual ob-

ject passes or doesn't pass a set mark, for example, the number of frogs that survive in a digitalis assay, or the number of positive tubes in a bacteriological experiment.

Statistics can best be applied to those assays where two or more doses of the standard are run simultaneously with two or more doses of the unknown.

Potency of an unknown is usually calculated as a certain per cent of the standard. It can thus be obtained by getting the antilog of the difference between the logarithms of the doses of the unknown and of a standard having the same response (since a quotient is a difference in terms of logarithms). It has been found that when the logarithm of the dose is plotted against some function of the response the result is a straight line.

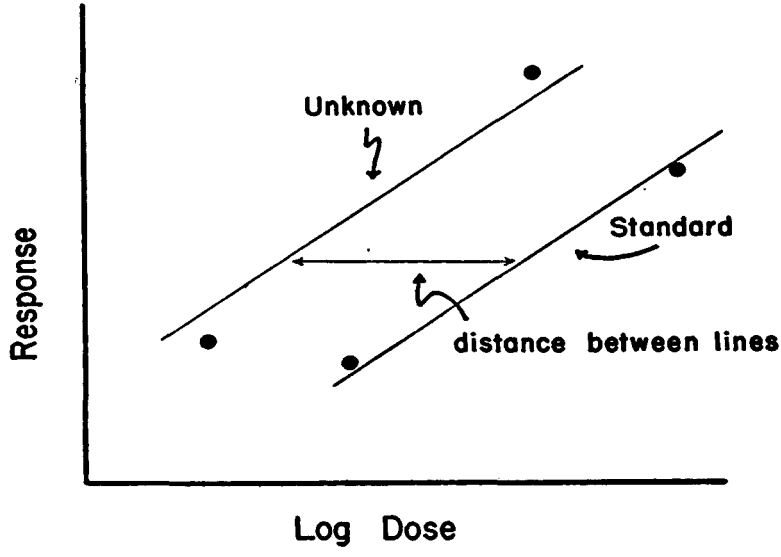

FIG. 1

Much has been written on this subject by various writers. Among the first were: Trevan (13), Gaddum (6), and Bliss (3).

It will suffice to say that, when the logarithm of the dose is plotted against some function of the response, two parallel straight lines are obtained (as shown in Figure 1), one for the standard material and one for the assay material (for which a dosage based on an assumed potency was used). Then, since the potency of the unknown is the antilog of the difference between the logarithms of the doses for standard and for unknown, the potency can be calculated from the horizontal distance between the lines. The parallel lines are usually fitted to the observations by least

squares (so-called because it mathematically minimizes the sum of the squares of the distances from the observed points to the calculated line). The calculated error of an assay is, then, some quantitative measure of the scatter of the observed points about the lines but depends also on the slope of the lines. At least two assumptions are made in this mathematical reasoning. The first is that the relationship between the log dose and the response is a straight line, and the second is that equal percentage increases in dose of standard and of unknown will give equal increases in response (*i.e.*, that the unknown has the same effect as the standard). Both these assumptions should be tested, and no assay run until they are known to be true within the range recommended for the assay.

The calculated error of an assay cannot estimate errors that cannot be shown to cause variations *within* the assay. Ordinarily, it cannot take into account the errors of weighing and measuring of the material (which are sizable in some assays, notably penicillin), nor can it take into account the differences between materials used in each laboratory, between samples of the lot to be assayed, or, most important, between assayists. The assayist *cannot* state, on the basis of the calculated error of the assay, that the lot assayed was, say, 650 ± 20 units per milligram. He *can* say that, if he repeated the assay many times using the same "made-up" solutions, this calculated error could be used to estimate the unavoidable variation in his own series of results.

In biological assays, as in chemical analyses, there are several types of errors (8). They can all be calculated in one way or another, but usually not from a single assay. (1) There is the error within which one assayist can check himself on the same "made-up" solutions or mixtures; (2) there is the error within which one assayist can check himself on the same lot of original material; and (3) there is the error within which one assayist can check another.

Rare indeed is the biological method that has been so standardized and checked collaboratively by assayists of long experience with the method, that the results of an assay can be duplicated as closely by another laboratory's assayist as they can be checked by the original assayist. Only in such exceptional cases will the error calculated from the data of a single experiment or assay be "the error of the method." Even then there is the danger that some assayist relatively inexperienced with the method will attempt to estimate the error of the method solely from his own data.

When the error calculated from the data of one experiment or one assay may be reliably used as the error of the method, the results from different laboratories are said to be "in a state of statistical control," that is, *any* laboratory can check the results of another laboratory as closely as it can check on its own results. This means that "Quality Control Methods" could be used on collaborative bioassay results. These methods are being adapted from those developed by Shewhart (10) and

explained in three publications of the American Standards Association (1). Quality control methods involve plotting the data on a graph with time (or experiment number) on the horizontal axis, and the magnitude of results on the vertical axis. The "control" is maintained by inserting on the chart the "control limits" which have been calculated from accumulated experience and drawn on the chart as parallel horizontal lines, as shown in Figure 2. When all the points fall within the limits, the results are said to be in a state of statistical control. When a dot falls outside the limits, trouble is indicated. An application of quality control methods to penicillin assays will be given elsewhere (9).

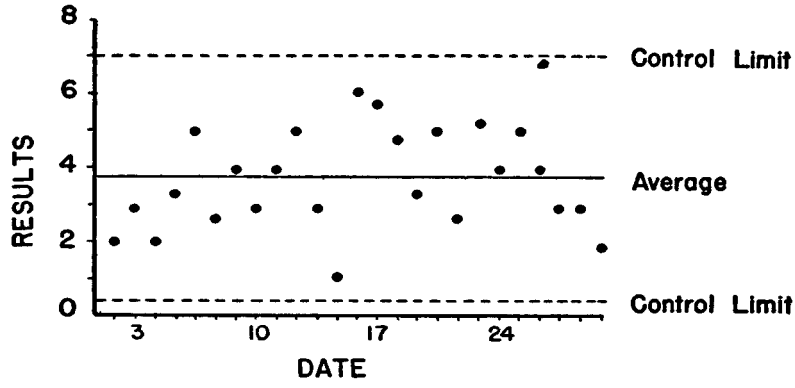

FIG. 2

A few more suggestions about how "Quality Control" can be used in biological work are offered in closing. Responses to a standard can be plotted on a control chart, even though there is a seasonal trend, since control limits can be drawn to have the same curvature as the trend. The weights of mature rats and the number of animals in each litter could be plotted on a control chart, to insure a continual supply of uniform animals and to spot any defects in diet and care that may be causing an untoward variation in the animals, or in the sizes of litters.

However, a long paper could be given on quality control alone, and the purpose of this paper has been merely to cover a few of the "high spots" of statistics as applied to biological experimentation and assay, to give a few references for further study if desired, and to point out pitfalls easily overlooked by the neophite while galloping along on the white charger "statistics."

SUMMARY

The use of a statistically designed experiment increases the precision and validity of an experiment.

Analysis of results should be carefully interpreted, and the assumptions made should be explicitly stated.

Assays can be evaluated in terms of potency and errors of the assay.

REFERENCES

(1) AMERICAN WAR STANDARDS: (a) "Guide for Quality Control," (b) "Control Chart Method of Analyzing Data," (c) "Control Chart Method of Controlling Quality during Production," American Standards Association, 29 West 39 St., New York City.

(2) ANDERSON, J. ANSEL, "Role of Statistics in Technical Papers" (given May 1944), *Transactions Am. Assoc. Cereal Chem.*, Vol. III, No. 2 (Jan. 1945).

(3) BLISS, C. I., "The Calculation of the Dosage-Mortality Curve," *Ann. Applied Biol.*, **22**, 134 (1935).

(4) FISHER, R. A., "Design of Experiments," Oliver and Boyd (1935).

(5) FISHER, R. A. and YATES, F., "Statistical Tables for Biological, Agricultural, and Medical Research," Oliver and Boyd (1943).

(6) GADDUM, J. H., "Reports on Biological Standards," Spec. Report Series, No. 183, Medical Research Council (1933).

(7) HARDING, T. SWAN, "They Understand Not One Another's Speech" (given May 1944), *Transactions, Am. Assoc. Cereal Chem.*, Vol. III, No. 2 (Jan. 1945).

(8) KNUDSEN, L. F., "The Value of Statistics in the Formulation of Chemical Methods," *This Journal*, **27**, 145 (Feb. 1944).

(9) KNUDSEN, L. F., and RANDALL, Wm. A., "Penicillin Assay and its Control Chart Analysis," *J. Bact.*, **50**, (Aug. 1945).

(10) SHEWHART, WALTER A., "Economic Control of Quality of Manufactured Product," Van Nostrand (1931).

(11) SIMON, LESLIE A., "An Engineers Manual of Statistical Methods," John Wiley & Sons (1941).

(12) TIPPETT, L. H. C., "Random Numbers," Tracts for Computers, Department of Applied Statistics, University College, Cambridge Univ. Press, London (1927).

(13) TREVAN, J. W., "The Error of Determination of Toxicity," *Proc. Roy. Soc.* (London), B, **101**, 483.

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