DR. FRANK THOMAS SHUTT, 1859-1940

FRANK THOMAS SHUTT

Frank Thomas Shutt was born in Stoke Newington, London, England, on September 15, 1859, a son of Denis Shutt, C. E., and Charlotte Cawthorne Shutt. After receiving private tuition in England, he came to Canada as a boy and was a pupil of Dr. William Hodgson Ellis at the School of Technology, Toronto. On removal of this school to Queen's Park to become the School of Practical Science of the University of Toronto, Dr. Ellis became Professor of Applied Chemistry and acted as Public Analyst. As his assistant for five years Shutt obtained valuable training and experience in analytical chemistry.

In 1885 he obtained his B.A. from the University of Toronto with first-class honors in chemistry, and he received his M.A. one year later. In 1914 he was awarded the D.Sc. (*honoris causa*). On the formation of the Dominion Experimental Farms System, with headquarters at Ottawa, he was chosen to be the first chemist, in 1887. In 1912 he received the title of Dominion Chemist and was also appointed Assistant Director. He served in this dual capacity until his retirement in 1933.

During his forty-six years of service Shutt made many valuable contributions to agriculture. Always interested in the practical side of farming, his guiding thought was the application of chemistry to the every-day problems of the farmer. He was a pioneer in Canada in establishing on a firm scientific basis the manurial value of clover and legumes. His investigations with fertilizers have gone far to bring about their rational and judicious use. In 1901 he published results of an extensive study of the "soft pork" problem, which has proved a valuable guide to all who are interested in pork and bacon production. His investigations of the qualities of Canadian-grown wheats were extensive and had much to do with the permanent establishment of Marquis wheat. His publications in scientific journals are numerous, and he frequently contributed articles of practical value to the agricultural press.

Shutt's association with the A.O.A.C. began in 1887, when he attended the fourth annual convention. In his first annual report, published in February, 1888, he refers to his visit to the Washington laboratories, which he described as in the basement of the building and at that time much too small for the number of chemists working and the amount of work in progress. In 1893 Shutt was appointed to represent the British Empire as professional juror on cereals at the World's Columbian Exposition, Chicago, and the results of his investigations were collated and prepared for publication by the late Dr. Wiley and issued in September, 1895, by the Department of Agriculture at Washington as Bulletin No. 45 of the Division of Chemistry. Throughout his career Shutt retained an active interest in the proceedings of the A.O.A.C., and his laboratory continued to cooperate in the collaborative investigations of the Association.

Dr. Shutt's achievements in scientific agriculture have been widely recognized. In 1935 he received the honour of the C.B.E. The Royal Society of Canada awarded him the Sir Joseph Flavelle medal, and he received the prize of the American Society of Agronomy for outstanding research. The Canadian Institute of Chemistry recognized his services by electing him to an honorary fellowship. He was a Fellow of the Chemical Society, the Royal Society of Canada, and the Institute of Chemistry of Great Britain and Ireland. He was elected to the Society of Public Analysts in 1916, and he was also a member of the Society of Chemical Industry, the American Chemical Society, the American Association for the Advancement of Science, and the Association of Official Agricultural Chemists.

On January 5, 1940, Dr. Shutt died at his home in Ottawa, but his many contributions to the agricultural chemistry of North America will remain a lasting tribute to his memory.

Apart from his profession, Dr. Shutt's main attachment was to his family.

He remained a bachelor. He was devoted to his mother and, on her death, to his sister, who took her place. For her and his brothers, his nephews and nieces, he was the center and chief tie of union of the family.

His life was enriched by a number of hobbies. He was fond of music; indeed, for several years he played the organ in an Ottawa church. In later years, he derived much pleasure from the gramophone and the radio. He was to the end an ardent photographer, and some of his work attained a high degree of excellence.

His was a thoroughly conservative nature. In politics he took no part, and in music, in art, in thought, and in manners he held to the old ways.

C. H. ROBINSON

SECOND DAY

TUESDAY—MORNING AND AFTERNOON SESSIONS—*Continued*

REPORT ON DRUGS

By L. E. WARREN (U. S. Food and Drug Administration Washington, D. C.), *Referee*

Owing to a combination of fortunate circumstances last year, the Association was able to close 12 topics, or over 50 per cent of the total number of 23 studied, but the large number of new subjects taken up this year did not permit such a fine record of closures. Of 25 topics in the research program this year, 4 have been sufficiently studied for the Referee to recommend the adoption of methods and discontinuation of the subjects.

The Referee is recommending that rhubarb and rhaponticum be discontinued because the associate referee is fully occupied with other duties.

Active revision of *Methods of Analysis* is to begin immediately after this meeting, and no changes in methods that have not already been made, or that have not been made before the close of this meeting, may be introduced into the book.

Book on Drug Analysis.—During the year but one important book on drug analysis has appeared in English. This is a third edition of the well-known treatise, "The Plant Alkaloids," by Thomas A. Henry. The third edition has been considerably enlarged and extensively rewritten. The chapters on the more important alkaloids describe the chemical and physical properties, occurrence, physiological actions, and methods of determination of the alkaloids and their salts. To those who have used the earlier editions, further introduction is unnecessary.

Microchemical Tests for Alkaloids.—This subject has been studied for many years, and methods for about 37 alkaloids have been adopted. This year the associate referee and his collaborators studied physostigmine and stovaine. No work was done on two others (coniine and cytisine) because material was not available. The associate referee recommends that the methods that he has developed for physostigmine and stovaine be adopted as tentative. The Referee concurs.

Last year the status of the microchemical tests for 28 alkaloids was advanced from tentative to official, first action. Eight of these drugs are of synthetic origin. This year the associate referees recommend that the status of the methods for these alkaloids, together with two more that were already official, first action, last year, be advanced to official, final action. The Referee concurs. The alkaloids in the proposed change of status are named below:

Aconitine	Codeine	Pilocarpine
Apomorphine	Ephedrine	Procaine
Arecoline	Ethylhydrocupreine	Quinidine
Atropine	Ethylmorphine	Quinine
Benzocaine	Homatropine	Scopolamine
Benzylmorphine	Hydrastine	Sparteine
Brucine	Hydrastinine	Strychnine
Caffeine	Hyoscyamine	Theobromine
Cinchonidine	Nicotine	Theophylline
Cinchonine	Papaverine	Yohimbine
Cocaine		

Microchemical Tests for Synthetics.—This subject has been under investigation for a number of years, and methods for 20 substances have been adopted as tentative. This year the associate referee studied the precipitative reactions for 14 chemicals that are used as hair dyes or that are chemically related to drugs used for that purpose. This project required considerable pioneering work. In general, he has found that the common alkaloidal reagents are precipitants for the hair dyes and that many of the precipitates are crystalline. He reported the results obtained but did not have time for collaborative tests. He recommends that the topic be continued with the view to collaborative study. The Referee concurs.

Last year the status of the microchemical tests for 18 synthetic substances was advanced from tentative to official, first action. This year the associate referee recommends and the Referee concurs that the status of 14 of these methods be advanced to official, final action. The synthetics in the proposed change in status are named below:

Acetanilid	Cinchophen
Acetophenetidin	Neocinchophen
Acetylsalicylic acid	Phenobarbital
Aminopyrine	8-Hydroxyquinoline
Antipyrine	Pyridium
Barbital	Salicylic acid
Benzoic acid	

The associate referee recommends that the status of the methods for the other 4 synthetics (amytal, dinitrophenol and its salts, methenamine, and triethanolamine) remain as official, first action.

Daphnia Methods.—Shortly after the last meeting the associate referee was appointed Scientific Adviser to the Kingdom of Thailand (Siam) and sailed for that country in December to be absent for several years. Reports were received from the associate referee and his assistant and some progress has been made. The Referee recommends that the topic be temporarily discontinued.

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

Guaiacol.—Two years ago a method in the literature, which depends on the determination of the alkoxyl group, was adapted by the associate referee to the determination of guaiacol, *This Journal*, **21**, 543 (1938). Last year the method was subjected to collaborative tests on known materials. The results are good, and the method was adopted for the determination of guaiacol. The method was published in *Methods of Analysis A.O.A.C.*, 1940, 608. However, the subject was continued with the view to determining guaiacol in mixtures. This year no work was done. Since the associate referee is occupied with other duties, the Referee suggests that the topic be discontinued.

Biological Testing.—No report was submitted. It is recommended that the subject be continued for another year.

Iodine Ointment.—A verbal report was submitted by the associate referee at the meeting. Some progress was reported. It is recommended that the topic be continued.

Acetophenetidin, Acetylsalicylic Acid, and Salol.—The associate referee and his collaborators studied two methods for the separation of acetophenetidin, acetylsalicylic acid, and salol. Both methods gave moderately good results in the hands of most of the collaborators. Others did not obtain such good results, particularly with Method II. The associate referee recommends that Method I be adopted as tentative and that the subject be closed. The Referee concurs.

Aminopyrine and Phenobarbital in Mixtures.—This topic has been under consideration for 3 years, and substantial progress has been made. Two methods have been suggested. In one the aminopyrine is held by dilute sulfuric acid and the phenobarbital is removed by a mixture of chloroform and ether. The solution is then made alkaline and the aminopyrine removed by chloroform. In the other method the phenobarbital is held by alkali and the aminopyrine removed by chloroform. The solution is then acidified and the phenobarbital removed by a chloroform-ether mixture. This year the associate referee has done no work. It is recommended that the subject be discontinued.

Elixir of Terpin Hydrate and Codeine.—This subject has been under investigation for several years. The chief difficulty has been the development of a satisfactory method for drying terpin hydrate. The associate referee has devised such a method, and the results of collaborative trials are good. The findings for codeine also are good. The associate referee recommends that the method be adopted as tentative. The Referee concurs and further recommends that the subject be closed.

Ointment of Yellow Mercuric Oxide.—This is a revival of a subject that was studied in 1931, *This Journal*, **15**, 409 (1932). This ointment is described in the U.S.P. XI and a method of assay is given. As the accuracy of the method has been questioned, a checking method seemed to be desirable. The associate referee applied a modification of the sulfide method

and obtained good results. He did not apply the tentative method for citrine ointment, *This Journal*, **22**, 96 (1939) to the ointment of yellow mercuric oxide. No collaborative work was done. The associate referee recommends that the Rupp method be tried next year. The Referee concurs with the added suggestion that the tentative method for citrine ointment be tried also.

Rhubarb and Rhaponticum.—Samples of rhaponticum were obtained last year and some preliminary studies were made. This year no work was done. It is recommended that the topic be continued for one more year.

Theophylline Sodium Salicylate.—In previous years, methods were developed for determining both theophylline and salicylic acid, but the results were not sufficiently accurate to justify the adoption of the methods. This year the associate referee has perfected gravimetric and volumetric methods for the assay both of theophylline and sodium salicylate. No collaborative work was done. The associate referee recommends that the subject be continued. The Referee concurs.

Mandelic Acid in Mixture.—Last year qualitative tests and a quantitative method for mandelic acid were adopted by the Association. However, the topic was continued with the view to the application of the method to mixtures. This year no report was submitted. The Referee believes that further consideration should not be given to the subject at this time.

Physostigmine Salicylate.—This is a new topic. The associate referee prepared a mixture of lactose and physostigmine salicylate and subjected it to the assay method recommended by the American Pharmaceutical Manufacturers' Association. The findings of the associate referee are good, but the results obtained by the collaborators are somewhat low. The associate referee recommends that the subject be studied further and that more collaborative work be done. He also recommends that the subject be expanded to include the study of ointments containing physostigmine. The Referee concurs.

Separation of Acetanilid and Salol.—This is a new topic. No report was received. It is recommended that the subject be discontinued.

Arecoline Hydrobromide.—This topic was assigned last year because areca products are used extensively in veterinary practice. Three methods were considered by the associate referee. Of these, steam distillation (1) and extraction with volatile solvents (2) were tried in a preliminary way, but no work was done with the silico-tungstic acid method (3). The associate referee recommends that the subject be continued. The Referee concurs.

Benzedrine.—This is a new topic. The name established by N. N. R. is amphetamine. The associate referee has been unable to obtain supplies of the base, but has used a steam distillation process on tablets of benzedrine sulfate. The liberated base is distilled into an excess of standard acid, followed by back titration. The results are not so good as desired.

The associate referee recommends that the topic be continued. The Referee concurs.

Plasmochine.—No report was submitted prior to the meeting. At the meeting a verbal report that indicated that some progress had been made was submitted. It is recommended that the topic be continued.

Hydroxyquinoline Sulfate.—No report was submitted. It is recommended that this topic be continued.

Pepsin.—No report was submitted on this new topic. It is recommended that the subject be continued.

Ipecac and Opium Powder (Dover's Powder).—This is a new topic, but no report was submitted by the associate referee. It is recommended that the topic be continued.

Nicotinic Acid.—Since this problem was assigned a year ago the Pharmacopoeia of the United States has described the product and provided an assay. However, the associate referee applied his efforts to the assay of the product in mixtures. He devised an ingenious sublimation method by which the acid is obtained nearly pure and weighed. The results from three collaborators are excellent and from two others moderately good. The associate referee is in doubt whether to recommend adoption of the method as tentative or to continue the study. The Referee recommends that the method be adopted as tentative and that the subject be closed. The method was adopted as tentative and published in *Methods of Analysis, A.O.A.C.*, 1940, 610.

Ephedrine in Jellies.—A method for the determination of ephedrine in inhalants was adopted as tentative in 1931, *This Journal*, **14**, 52 (1931). This method was designed for the determination of ephedrine alkaloid in oil solutions. Preparations now on the market contain ephedrine salts dissolved in mucilages, jellies, etc. The tentative method is not applicable to solutions of this nature. Early in the year, the Referee recommended that an associate referee be appointed to study this subject. This was done and a report has been received.

The associate referee found that by a simple modification of the tentative method for ephedrine in inhalants, it could be applied to the assay of ephedrine salts in jellies. He recommended that the method be amended to provide a temperature not above that of the room while the ethereal solutions of the alkaloid are being evaporated. The Referee concurs.

Purification of Caffeine.—During past years several complaints were received that the method, official, first action, *Methods of Analysis, A.O.A.C.*, 1935, 544, **7(a)**, for the purification of caffeine, resulted in losses. The method consists in dissolving the impure caffeine in diluted sulfuric acid, precipitating with iodine solution, and regenerating the caffeine from the precipitate. Last year an associate referee was appointed to study the subject. Comprehensive tests by the associate referee and his collaborators demonstrated that the criticisms were justified. Losses of as

much as 20 per cent were reported. By a simple modification, the method was made to yield satisfactory results. The associate referee recommends that the present method be amended to add larger specified quantities of acid before precipitating the caffeine and that the amendment be adopted as tentative. The Referee concurs.

Since the original method is official, first action, the Referee recommends that the amendment just adopted as tentative be adopted as official, first action, and that the amended method be adopted as official, final action, under suspension of the rules.

REVISION OF METHODS

Procaine.—Method II for procaine (p. 574, 90) directs that the chloroformic solution of the alkaloid be evaporated in a weighed beaker, but no directions for weighing the residue are given. The Referee recommends that after the expression, " . . . slightly volatile at 100°," the following expression be inserted: "Add a few ml. of anhydrous ether and evaporate while holding the container in an inclined position. Dry the residue at 60° and weigh."

Barbital and Phenobarbital.—The Referee recommends that the amendment to the official method for barbital and phenobarbital, official, first action, which reads, "Determine the melting point to check the purity of the residue," be adopted as official, final action.

Ephedrine in Inhalants.—The Referee recommends that the method for the determination of ephedrine in inhalants, 557, be amended by deleting the expression, "on a steam bath with moderate heat," from the next to the last line in par. 42.

Aminopyrine (Pyramidon).—The Referee recommends that the official method for the determination of aminopyrine (pyramidon), 573, 87, be amended by the substitution of the expression, "H_2SO_4," for the expression "HCl" in the second and third lines.

Salicylic Acid in Presence of Other Phenols.—The associate referee recommends that the status of the above-named method, 570, be advanced from tentative to official, first action. The Referee concurs.

Hypophosphites in Sirup.—Last year the Association adopted a method for the determination of hypophosphites in sirup. This year the associate referee recommends that the method be advanced to official, first action. His reasons appear to be sound and the Referee concurs.

Calomel in Calomel Ointment.—Calomel ointment is described in the N.F. VI, but no assay is provided. A method was adopted, as tentative, by the A.O.A.C. in 1930, *This Journal*, 14, 312 (1931). The associate referee recommends that the status of this method be advanced to official, first action. The Referee concurs.

Monobromated Camphor, Method II.—This method has been in the ten-

tative stage for some years. No criticisms having been received, the associate referee recommends that it be advanced to official, first action. The Referee concurs.

Tetrachlorethylene in Mixtures.—The associate referee reports that no criticisms have been received and that the method is a standard one for chlorine. He recommends that the status of the method be advanced to official, first action. The Referee concurs.

Cod Liver Oil Emulsions.—A method for the assay of cod liver oil emulsions was adopted last year, *This Journal*, **22**, 96 (1939). The associate referee recommends that the method be made official, first action. The Referee questions the advisability of this action at this time.

Santonin in Mixtures.—Last year the tentative method for santonin in mixtures, 129, was made official, first action, but the method in paragraph **128** was left unchanged. The associate referee recommends that this method **(128)** now be made official, first action. The associate referee also suggests that both methods be combined under one heading "Santonin in Mixtures" and designated as Method I and Method II. The Referee concurs and suggests that both methods be made official, final action, the latter under suspension of the rules.

Emetine Hydrochloride in Tablets.—The method for emetine hydrochloride in tablets, **35**, is now official (first action). The associate referee recommends that this method be made official (final action). The Referee concurs.

Apomorphine in Tablets.—Official, first action, **67.** The associate referee recommends that the method for the assay of these tablets be made official, final action. The Referee concurs.

Antipyrine and Caffeine.—A method for the determination of antipyrine and caffeine in mixtures has been official, first action, for some years, 553. In general, it has given satisfaction. The Referee recommends that the status of this method be advanced to official, final action.

Iodoform Ointment.—Last year the status of the methods for iodoform and iodoform gauze was advanced from tentative to official, first action, *This Journal*, **22**, 100 (1939). Through an oversight the method for iodoform ointment was not included in the recommendation. The Referee now recommends that the status of the tentative method for iodoform ointment be advanced from tentative to official, first action, *Methods of Analysis, A.O.A.C.*, p. 594.

The Referee further recommends that the method for the assay of iodoform ointment just adopted as official, first action, be adopted as official, final action, under suspension of the rules.

Iodoform Gauze.—The Referee recommends that the methods for iodoform and iodoform gauze, official, first action, *This Journal*, **22**, 100 (1939), be advanced to official, final action.

Morphine in Sirups.—No adverse comments on this method have been received, but the associate referee has made no recommendation concerning it. The Referee recommends that the status be advanced to official, final action.

Camphor.—The method for the determination of camphor, 560, **51,** was amended by the addition of the parenthetical phrase, "Not applicable to synthetic camphor," official, final action.

Santonin.—The methods for the determination of santonin, 587, **128** and **129,** were adopted as official, final action, Method **129** under suspension of the rules.

Swelling Factor of Psyllium.—A tentative method for estimating the swelling value of psyllium was adopted in 1935, *This Journal,* **19,** 104 (1936). The National Formulary VI describes psyllium and provides for an evaluation of its swelling properties. The advisability of deleting the A.O.A.C. method should be considered.

NEW SUBJECTS

Theobromine and Phenobarbital.—Mixtures of theobromine and phenobarbital are on the market. No method has been adopted for the assay of such preparations. The Referee recommends that theobromine-phenobarbital mixtures be studied.

Quinine Ethyl Carbonate (*Euquinine*).—This drug is marketed chiefly in tablets. It is described in the U.S.P. XI, but no assay is included. It is recommended that quinine ethyl carbonate be studied.

Chlorobutanol.—Last year the Association adopted a method for the assay of chlorobutanol that involved distillation and subsequent decomposition of the chlorine compound by alcoholic potassium hydroxide. Later a member of the American Pharmaceutical Manufacturers' Association submitted a method by which the distillation procedure and the pressure bottle were eliminated. This information was transmitted to the associate referee and to one member of Subcommittee B, but no decision was reached. The Referee recommends that the subject be reopened in order to study the simplified method.

Prostigmine.—Recently a synthetic having structure and uses similar to physostigmine has come into use in the diagnosis of certain muscular diseases. Its chemical and physical properties are described in the literature, but no methods of assay were found in the compendiums by the Referee. It is recommended that prostigmine be studied.

Magnesium Trisilicate.—During the past three years magnesium trisilicate has come into considerable use in the treatment of gastric ulcer. The literature concerning the chemistry and assays of this complex compound is limited. This Association has made no study of this preparation. The Referee recommends that an associate referee be appointed to study this subject.

Lobeline.—Lobeline salts are being used to considerable extent in medicine. The Referee recommends that lobeline and its salts be studied.

Compound Ointment of Benzoic Acid (Whitfield's Ointment).—This ointment is described in the N.F. VI but no assay is provided. Assay methods for similar products are described in the literature. The Referee recommends that this subject be studied.

Dilaudide Hydrochloride.—In recent years dilaudide hydrochloride has come into use as a substitute for morphine. It is recommended that microchemical tests for dilaudide be studied.

Benzocaine.—Ethylaminobenzoate (benzocaine) is described in the Pharmacopoeia of the United States, but no assay is provided. It occurs on the market either alone or mixed with other drugs. An assay method is needed. It is recommended that benzocaine be studied.

Sulfapyridine.—Sulfapyridine has come into extensive use during the past year in the treatment of pneumococcic infections. Neither the Pharmacopoeia of the United States nor the National Formulary describes it. The Referee recommends that sulfapyridine be studied.

Methylthionine Chloride (Methylene Blue).—The Association has adopted as official a method for the assay of this substance. Last year the Referee called the perchlorate method for the assay of methylthionine chloride to the attention of the associate referee. After making a brief study of the matter, he decided that no action by the Association was necessary. Recently the perchlorate method was adopted by the U. S. Pharmacopoeia XI (Supplement II, p. 75). The Referee recommends that the status of the official method for methylthionine chloride be reduced to official, first action. The associate referee recommends that the subject be reassigned for further study. The Referee considers that there is little need for this at the present time.

Aminopyrine in Mixtures.—Owing to the introduction of new drugs into medicine and the continued practice of combining two or more in a single dosage form, the analysis of medicinal preparations is a problem of constantly increasing complexity. The A.O.A.C. recognized the importance of this phase of the work some years ago and already methods for the analysis of several mixtures have been adopted.

A recent examination revealed that aminopyrine is on the market in mixtures with one or more of the following drugs: acetanilid, acetophenetidin, acetylsalicylic acid, antipyrine, caffeine, barbital, cinchophen, phenobarbital, sodium bicarbonate, sodium benzyl succinate, sodium salicylate, strontium salicylate, and various botanical extractives. At present an associate referee is studying aminopyrine and phenobarbital in mixtures. The Referee is of the opinion that mixtures of aminopyrine and other drugs should be studied. He recommends that aminopyrine, acetophenetidin, and caffeine be studied.

REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS

By CHRIS K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In accordance with the Referee's communications last year, that quinine ethyl carbonate, physostigmine, and stovaine be included for study, the subject was continued.

In the preliminary work, the test for physostigmine with the new reagent containing lead iodide described by H. G. Wagenaar[1] yielded characteristic radiating serrated plates. According to Amelink[2] gold-chloride reagent forms dendritic or tree-like crystals with stovaine in neutral or acid solution.

Since quinine ethylcarbonate is only slightly soluble in water, and readily soluble in dilute acids, as stated in the U.S.P., it responded to the test for quinine with an excess of the reagent disodium phosphate, forming sheaf-like crystals.[3]

Directions for the tests, control specimens of physostigmine (labeled "eserine sulphate") and stovaine, also samples for identification marked Nos. 1 and 2, were sent to the collaborators. The material for the control was considered sufficiently pure for the work.

The tests were published in *Methods of Analysis, A.O.A.C.*, 1940, 632.

COLLABORATORS' RESULTS AND COMMENTS

John R. Matchett, Bureau of Narcotics, Treasury Dept., Washington, D. C.—

Physostigmine and lead iodide.—Crystals formed as described in the 1:100 solution, starting at the edge of the drop and continuing to form throughout the drop for 15 minutes. Unknown solution No. 2 was readily identified as physostigmine.

Stovaine and gold chloride.—When the method was used as described, crystals formed very slowly in the drop. Better results were obtained when a drop of concentrated HCl was added to the drop of alkaloidal solution followed by a drop of reagent. The crystals formed faster and appeared more definite in form. The crystals should be useful for identification since they differ markedly from those formed by cocaine or novocaine. Unknown solution No. 1 was identified as stovaine.

H. Robert Bond, U. S. Food and Drug Adm., Kansas City, Mo.—

Stovaine.—No. 1 with $AuCl_3$ reagent + HCl. Crystals long, tooth-edged, branched.

Physostigmine.—No. 2 with PbI_2 reagent. Radiating serrated crystals.

Charles C. Fulton, Treasury Department, Internal Revenue Service, St. Paul, Minn.—

Stovaine.—$AuCl_3$ or $AuCl_3$ with HCl gives the best test. Stovaine gives good crystals with a number of other reagents, including the new PbI_2 reagent.

Physostigmine.—The best test, in my opinion, is with $AuCl_3$ in concentrated HCl (applied to the aqueous solution). However, the test with the new PbI_2 reagent I rank as second best. The crystals it gives are very satisfactory for identification.

Formerly I have used PbI_2 reagent, made by dissolving Pb acetate in a very concentrated NaI solution. This gives crystals with both stovaine and physostigmine; however, it is a very inconvenient reagent, as an excess is required to avoid

[1] *Pharm. Weekblad*, 76, 276 (1939).
[2] Schema zur Microchemischen Identification von Alkaloiden, 1934.
[3] *Methods of Analysis, A.O.A.C.*, 1935, 604.

precipitation of PbI_2 on dilution, and the crystals produced are very small. The new reagent, PbI_2 in concentrated potassium acetate solution, is less sensitive, but superior in all other respects.

James H. Cannon, U. S. Food and Drug Adm., St. Louis, Mo.—

Solution No. 1.—Stovaine.

Solution No. 2.—Physostigmine.

Both tests yielded good crystals readily.

SUMMARY

The alkaloids were identified correctly by the collaborators.

The test for stovaine was modified to include the observation made by Machett that better results were obtained by adding one drop of concentrated hydrochloric acid to the alkaloidal solution followed by a drop of the gold-chloride reagent.

Material for tests for coniine and cytisine were not available.

RECOMMENDATION[4]

It is recommended—

(1) That the microchemical tests for physostigmine and stovaine be made tentative.

(2) That study of coniine and cytisine be discontinued until material is available.

REPORT ON MICROCHEMICAL TESTS FOR SYNTHETICS

By IRWIN S. SHUPE (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Paraphenylenediamine and 2,5 diaminotoluene were recommended as subjects for this year's A.O.A.C. report. These diamines and many other amino compounds have been used or proposed for use in the manufacture of hair dyes. For comparative purposes, fourteen of these related compounds were studied as a group. This is far from a complete list of hair dye materials, but it includes many of those used at present in this country.

The identity of the various compounds used in the tests was established by comparing the melting points of the free bases and acetyl derivatives with constants given in the literature. With the exception of diaminoanisole, there seems to be no doubt as to their identity. Deshusses[1] states that he found discrepancies in samples of 2,5 diaminoanisole purchased in Switzerland; and that the free base melted at 52°–53° C. and 74°–75° C. for two different samples. The free bases that were used in these tests, purified by vacuum distillation, melted at 62°–63° C. for the 2,4 diaminoanisole, and at 103°–104° C. for the 2,5 diaminoanisole. Corresponding acetyl derivatives prepared from the purified bases melted at 203°–204° C. for the 2,4 and at 224°–225° C. for the 2,5 compound. Beilstein[2] gives the

[4] For report of Subcommittee B and action by the Association, see *This Journal*, 23, 55 (1940).

[1] *Mitt. Lebens. Hyg.*, 30, 1/2, 10 (1939).

[2] Beilstein, Handbuch der Organischen Chemie, Suppl. Vol. 13, 4th ed. (1933), p. 204.

melting point of the 2,4 base as 67°–68° C. Griebel and Weiss[3] give 103°–104° C. for the melting point of 2,5 diaminoanisole. Further work on the identity of these compounds is under way.

For microcrystalline identification tests, Rosenthaler[4] proposes cobalt and mercury ammonium thiocyanates as reagents for paraphenylenediamine and 2,5 diaminotoluene. Field and Cannon[5] propose the use of acetic anhydride and Carol[6] uses silicotungstic acid as reagents for paraphenylenediamine.

In addition to Rosenthaler's[4] cobalt and mercury complexes, the cadmium, nickel, and zinc thiocyanates were found to produce crystalline precipitates with the free bases, as shown in Table 1. Salts of the bases did not respond to the tests, apparently because of their acidity.

The reagents were prepared by adding an equal volume of a 5 per cent solution of the metallic salts to a 10 per cent solution of ammonium thiocyanate.

TABLE 1.—*Reactions with various reagents*

AMINES	REAGENTS				
	COBALT NITRATE + NH_4SCN	MERCURIC CHLORIDE + NH_4SCN	CADMIUM CHLORIDE + NH_4SCN	NICKEL ACETATE + NH_4SCN	ZINC CHLORIDE + NH_4SCN
p-Phenylenediamine	c	c	c	c	c
m-Phenylenediamine	a	a	c	a	c
o-Phenylenediamine	c	a	—	c	—
2,5 Diaminotoluene	c	a	c	a	a
2,4 Diaminotoluene	—	—	c	—	—
3,4 Diaminotoluene	—	a	—	c	c
2,4 Diaminoanisole	a	a	a	a	c
2,5 Diaminoanisole	c	a	c	c	a
p-Aminophenol	—	—	—	—	—
o-Aminophenol	—	—	—	—	—
4-Aminodiphenylamine	—	—	—	—	—

"a" = amorphous precipitate, "c" = crystalline, and "—" = no precipitate.

Reactions with several general alkaloidal reagents and with acetic anhydride are outlined in Table 2. Wagner's, Mayer's and Marmé's reagents were prepared as described in *Methods of Analysis, A.O.A.C.*, 1935, 602. The tests with acetic anhydride were made by adding a very small drop of the acetic anhydride to a drop of a water solution of the amine. The other reagents were 5 per cent solutions in water. With the exception of acetic anhydride, the tests were made by adding about 1 mg. of the

[3] *Z. Untersuch. Lebensm.*, 67, 86 (1934).
[4] *Mikrochemie, N. F.*, T, 15, 215 (1937).
[5] *This Journal*, 23, 717 (1940).
[6] *Ibid.*, 821.

powdered amine to a drop of the reagent and stirring slightly to aid solution.

TABLE 2.—*Reactions with general alkaloidal reagents and acetic anhydride*

AMINES	REAGENTS								
	PLATINIC CHLORIDE	WAGNER'S	MAYER'S	MARMÉ'S	SILICOTUNGSTIC ACID	PHOSPHOTUNGSTIC ACID	MERCURIC CHLORIDE	POTASSIUM OXALATE	ACETIC ANHYDRIDE
p-Phenylenediamine	c	c	c	c	c	a	c	c	c
m-Phenylenediamine	c	a	—	a	c	c	a	—	—
o-Phenylenediamine	c	c	—	c	a	c	c	c	c
2,5 Diaminotoluene	a	c	—	—	c	a	—	c	—
2,4 Diaminotoluene	c	a	—	—	c	c	—	—	—
3,4 Diaminotoluene	—	a	—	—	a	c	c	c	—
2,5 Diaminoanisole	c	a	—	c	c	a	c	c	—
2,4 Diaminoanisole	a	a	a	a	c	a	a	c	—
4 Aminodiphenylamine	a	c	—	—	a	c	c	c	c
p-Aminophenol	—	—	—	—	—	c	—	—	—
m-Aminophenol	a	c	—	—	—	—	a	—	—
o-Aminophenol	a	a	—	—	—	—	a	—	—
2,4 Diaminophenol Hydrochloride	c	a	—	—	c	c	—	c	—
p-Methylaminophenol Sulfate	—	c	—	—	a	c	—	—	c

"a"=amorphous precipitate, "c"=crystalline, and "—"=no precipitate.

All the reagents used, except phosphotungstic acid, produce crystalline precipitates with paraphenylenediamine, and 5 of the reagents produce crystalline precipitates with 2,5 diaminotoluene. Platinic chloride, Wagner's reagent, silicotungstic acid, and acetic anhydride are considred especially useful for characterizing these two diamines.

It is recommended[7] that the study of microchemical methods for the diamines and related compounds be continued and that samples of the more commonly used diamines be submitted for collaborative study.

REPORT ON DAPHNIA METHODS

By ARNO VIEHOEVER (Department of Science, Ministry of Economic Affairs, Bangkok, Thailand), *Associate Referee*

The application of daphnia as a testing animal or biological reagent was continued. Results of this work have been published as follows:

1. The Comparative Physiological Action of Benzedrine (Amphetamine) and Derivatives on Daphnia Magna, by Arno Viehoever and Isadore Cohen in *Am. J. Pharm.*, **110**, 526, 32 (1938).

2. Visualization of Drug Action, etc., by Arno Viehoever, in *Am. J. Pharm. Education*, **3**, 68–70 (1939).

3. Physiological Evaluation of Veratrum Viride and Veratrum Album, by Arno Viehoever and Isadore Cohen in *Am. J. Pharm.*, **111**, 86 (1939).

[7] For report of Subcommittee B and action by the Association see *This Journal*, 23, 56 (1940).

The following interesting conclusions were drawn from the last research:

1. A physiological method for the comparative evaluation of the toxicity of preparations of *Veratrum viride* and *Veratrum album* utilizes *Daphnia magna*, the transparent crustacean, as a test animal.

2. The action of the extracted veratrum principles upon the locomotion of standardized daphnia is so marked that the degree of impairment of this function (which is also related to the degree of internal depression) can be used quantitatively in comparing the potency of Veratrum preparations.

3. The impairment of internal organs is shown most strikingly in the depression of the cardiac and respiratory systems, which is of equally important diagnostic value.

4. Preparations of *Veratrum viride* made under like conditions from the same source of material show uniform potency.

5. A preparation of *Veratrum album* made under similar conditions revealed a potency at least 2.5 times greater than the preparations made from *Veratrum viride*, and a potency of at least 10 times that of a certain *Veratrum viride* preparation made under different conditions.

6. This varying potency has been verified by toxicity tests made upon the albino rat, guinea pig, and rabbit.

7. The depressant action of *Veratrum viride* and of *Veratrum album* on the locomotory, cardiac, and respiratory systems of *Daphnia magna* has been corroborated with the albino rat, guinea pig, and rabbit. For the precise measurement of cardiac depression in the rat, the newly devised stethographone was used.

In addition to satisfactory experimental work with daphnia carried out in the United States by various workers, as Arthur M. Banta and coworkers of the Carnegie Institution of Washington and Brown University, and Dr. Med Boericke, Professor of Pharmaco-Therapy of Hahnemann Medical College and Editor of the Hahnemannian Monthly, published by this college in Philadelphia, who presented his results before the Hahnemann Medical Society with demonstrations this summer in Atlantic City, and by European workers, quoted recently in Chemical Abstracts, the associate referee's former student, Mr. Sokoloff, proved in research with daphnia also, carried out with Dr. Perlman in the Jewish Hospital of Philadelphia, that nicotine is transmitted with the breast milk of smoking mothers. The method suggested by the associate referee for the biological assay of toxic substances was used. Komol Pengsritong, Medical Officer of the Dept. of Science here, verified the usefulness of daphnia for the testing of the cathartic action of a local variety of *Aloe vera*. Other domestic drugs, tested successfully here, include the croton seed, castor bean, and the seed of *Jatropha curcas*, the toxic latex of two *Euphorbia* species, the toxic seed of *Pachyrhizus* with edible roots, the tubers of various species of *Stemona*, a promising insecticide, and others to be mentioned later. Quantitative experimental evidence will be submitted when completed.

It is suggested that the work be continued.

PROGRESS REPORT ON DAPHNIA METHODS

By ISADORE COHEN (24 S. 34th St., Philadelphia, Pa.),
for ARNO VIEHOEVER, *Associate Referee*

The past year has witnessed the further introduction of the daphnia methods into industry for control purposes; for the evaluation of phar-

macological and toxic action of new substances, including laxative agents; and, finally, as an addition to clinical research procedures.

Until January 1939, Dr. Viehoever and the writer made available the daphnia methods of evaluation and standardization of pharmaceutical products and new organic synthetics to a number of industrial firms. This year, the writer continued part of this service.

The suggested tentative method for the biological assay of toxic substances, *This Journal*, **22**, 717 (1939), was extended to the determination of the evaluation of powders of *Veratrum viride* with the following slight modification in procedure (*Am. J. Pharm.*, **111**, 86 (1939)):

> Extract 1 gram of each lot of veratrum powder in 100 ml. of the culture medium in which the test daphnia are grown for 15 minutes, with vigorous, continued shaking. Add 500 ml. of each filtrate to 50 specified daphnia in 50 ml. of culture medium contained in the specified test jars, to make a 0.5% aqueous test solution. (A line drawn through the midlevel will provide two zones for observing the activity of the test daphnia.) On the basis of the debility shift, select the weakest lot and use as a comparative reference standard. Dilute the other test solutions until their action upon the test daphnia matches that of the comparative reference standard. Assigning unity to the comparative reference standard, then the quantitative differences between the test lots are equal to the ratio derived between the concentration of the reference standard and the matching concentrations of the other test solutions. A sufficient amount of veratrum powder found to be satisfactory should be suitably stored for subsequent use in the control of new lots, evaluation of toxicity, etc.

The writer has cooperated with H. H. Perlman of the Jewish Hospital' Philadelphia, Pa., in conducting preliminary experiments on the detection of nicotine in high dilutions, especially in the concentrations transmitted through human breast milk during lactation. The experimental data obtained assured the feasibility of the use of *Daphnia magna* in this connection. The technical work, which is nearing successful completion, has been carried out by Nathan H. Sokoloff, trained in the daphnia methods by Arno Viehoever and the writer.

Work now in progress is represented by investigations dealing with the use of daphnia in the study of estrogens and androgens, various components of the vitamin B complex, in the evaluation of chlorine-liberating substances, and general refinements in the biological assay of toxic substances.

The courtesy of the Trustees and the Director, Leonard G. Rowntree, of the Philadelphia Institute for Medical Research, is acknowledged in providing the facilities necessary for the prosecution of these activities.

No report on ergot alkaloids was given by the associate referee.

No report on guaiacol was given by the associate referee.

No report on biological testing was given by the associate referee.

No written report on iodine ointment was given by the associate referee.

REPORT ON THE SEPARATION OF ACETYLSALICYLIC ACID, ACETOPHENETIDIN, AND SALOL

By DONALD C. GROVE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Tablets containing acetylsalicylic acid, acetophenetidin, and salol are sold by several pharmaceutical manufacturers; therefore, it is desirable to have a quantitative method for the separation of these ingredients.

As *Methods of Analysis, A.O.A.C.*, 1935, 549, contains a satisfactory method for the analysis of mixtures of acetophenetidin and salol, the problem resolved itself into a method for separating the acetophenetidin and salol together from the acetylsalicylic acid.

Hitchens[1] presented experimental data showing that acetylsalicylic acid could be satisfactorily separated from a number of medicinal ingredients by means of sodium bicarbonate. Grove, *This Journal*, **22**, 91 (1939), also successfully separated acetylsalicylic acid from acetophenetidin and caffeine by this method.

Method I, as sent to the collaborators, was essentially the separation of the acetylsalicylic acid from the other ingredients by means of cold sodium bicarbonate solution, acidification of the sodium bicarbonate solution, and subsequent extraction of the liberated acetylsalicylic acid with chloroform. The acetophenetidin and salol were then determined by the A.O.A.C. alkaline hydrolysis method, *Methods of Analysis, A.O.A.C.*, 1935, 549, **19.**

The details of method I are as follows:

METHOD I

REAGENTS

(a) *Sodium bicarbonate solution.*—Prepared fresh. To 3 grams of $NaHCO_3$ add 45 ml. of water previously cooled to 15° C. or lower. Stir until dissolved and add 2 or 3 drops of dilute HCl.

(b) *Standard bromide-bromate solution (0.1 N bromine solution).*—Dissolve 3 grams of $KBrO_3$ and 12 grams of KBr in water and dilute to 1 liter. Standardize against 0.1 *N* $Na_2S_2O_3$ in the usual manner.

(c) *Standard sodium thiosulfate solution.*—0.1 *N* $Na_2S_2O_3$.

(d) *Sodium hydroxide solution.*—2.5% w/v NaOH.

DETERMINATION

(The determination of acetylsalicylic acid must be carried out as soon as possible to prevent any hydrolysis in the $NaHCO_3$ solution.)

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[1] *J. Am. Pharm. Assoc.*, **24**, 1084 (1934).

Acetylsalicylic acid.—Weigh 0.5–1.0 grams of the powdered tablets, transfer to a separator containing about 10 ml. of water cooled to 15° C. or lower, and shake thoroughly. Add 15 ml. of the cooled $NaHCO_3$ solution slowly to prevent mechanical loss due to effervescence and immediately extract with successive portions of $CHCl_3$. Wash each portion of $CHCl_3$ through a second separator containing 2 ml. of the $NaHCO_3$ solution and filter through a funnel containing a pledget of cotton moistened with $CHCl_3$. (Extraction is complete when a final shakeout evaporated to dryness leaves a negligible residue. Usually 5 extractions with about 30 ml. portions of $CHCl_3$ are sufficient.) Set aside the combined $CHCl_3$ extracts containing the acetophenetidin and salol for later treatment. Transfer the wash water in the second separator to the solution in the first separator, rinsing several times with small portions of water. Acidify the combined $NaHCO_3$ solution with HCl (1+1) and extract the acetylsalicylic acid by shaking with successive portions of $CHCl_3$, filtering each portion through a funnel containing a pledget of cotton moistened with $CHCl_3$. (Usually 5 extractions are sufficient.) Evaporate the combined $CHCl_3$ extracts on a steam bath with the aid of a fan or gentle air blast until the volume is about 10 ml. Transfer to a suitable small tared container with the aid of $CHCl_3$ and evaporate to dryness by means of a fan or gentle air blast without heat. Dry in a desiccator overnight and weigh as acetylsalicylic acid. (If desired, the extracted acetylsalicylic acid may be checked by the A.O.A.C. double titration method or by the bromination method.)

Acetophenetidin and salol.—Evaporate the combined $CHCl_3$ solutions containing the acetophenetidin and salol to dryness by means of a gentle air blast or fan without heat. Dissolve the residue in a few ml. of ether and again evaporate to dryness. Treat the residue as directed in the A.O.A.C. method, *Methods of Analysis*, page 549, 19(a), beginning "Add 10 ml. of 2.5% NaOH solution." In the determination of the salol, the following modification will be necessary: Combine the alkaline salol solutions, freed from the acetophenetidin, into a volumetric flask and make to volume with water. An aliquot of this solution, containing a quantity of salol not exceeding 0.08 gram, is then taken for bromination.

Salkover[2] reported a method for the separation of salol from acetanilid and also from acetophenetidin that depends on the relatively great solubility of salol in petroleum benzin and the relative insolubility of the other substances in that solvent. He did not present any analytical data in substantiation of his method. The solubility of salol in petroleum benzin is 1 part of salol per 1.1 parts of solvent; acetophenetidin, 1 in 10,200; and acetylsalicylic acid, 1 in 21,560.

A method was tried, therefore, based upon the removal of the salol from the acetylsalicylic acid and acetophenetidin by means of petroleum benzin, and separation of the two latter ingredients by means of sodium bicarbonate.

The details of Method II as submitted to collaborators are as follows:

METHOD II

REAGENTS

Same as those used in Method I.

DETERMINATION

Salol.—Weigh 0.5–1.0 gram of the powdered tablets into a small beaker. Treat with 10 ml. of petroleum benzin (b.p. 30°–65° C.), using a small stirring rod to stir

[2] *Am. J. Pharm.*, 88, 484 (1916).

TABLE 1.—*Collaborative results with Method I (per cent)*

Sample A

COLLABORATOR	ACETYLSALICYLIC ACID		ACETOPHENETIDIN		SALOL	
	FOUND	AVERAGE RECOVERY	FOUND	AVERAGE RECOVERY	FOUND	AVERAGE RECOVERY
H. H. Shull McNeil Lab. Philadelphia	33.17 33.20	99.6	33.55 33.50	100.6	32.37 32.14	96.8
F. H. Hedger* C. Pfizer & Co. New York	33.20	99.6	31.80	95.4	35.00	105.0
O. C. Kenworthy Food & Drug Adm. New York	33.34 33.50	100.3	33.91 33.70	101.4	32.87 33.17	99.1
R. L. Herd Food & Drug Adm. Washington	33.42 33.27	100.1	33.53 33.62	100.8	32.57 32.65	97.8
D. C. Grove Food & Drug Adm. Washington	33.73 33.40 33.10 33.40	100.2	33.57 33.53 33.30 33.33	100.3	32.95 32.70 33.25 33.25	99.1
C. F. Bruening Food & Drug Adm. Baltimore	32.57 32.73	98.0	33.32 33.48	100.2	34.10 34.05	102.2
Average	33.24	99.7	33.53	100.6	33.01	99.0
			Sample B			
H. H. Shull	28.67 28.62	98.8	29.28 29.18	100.8	27.55 28.11	96.5
F. H. Hedger	28.20	97.2	29.00	100.0	29.30	101.0
O. C. Kenworthy	28.42 28.37	97.9	29.25 29.38	101.1	30.21 29.91	103.7
D. C. Grove	28.63 28.85	99.1	28.77 28.83	99.3	29.20 29.31	100.9
C. F. Bruening	27.98 28.08	96.7	28.68 29.15	99.7	30.44 29.60	103.5
Average	28.42	98.0	29.06	100.2	29.29	101.0

* Figures not included in the average for Sample A.

the mixture, and decant the petroleum benzin through a 9 cm. filter paper, receiving the filtrate in a 100 ml. beaker. Repeat the washing of the acetophenetidin and acetylsalicylic acid in the beaker and filter with small portions of petroleum benzin until the volume of the filtrate measures about 75 ml. Allow the insoluble material in the beaker and on the filter to dry spontaneously and reserve for the determination of acetophenetidin and acetylsalicylic acid. The petroleum benzin solution is evaporated to apparent dryness by means of a gentle air blast or fan without heat. Add 10 ml. of the NaOH solution and heat for 5 minutes on a steam bath. Cool, and transfer to a 100 ml. volumetric flask and make to volume with water. Take an aliquot of this solution representing not more than 0.08 gram of salol and brominate as in the A.O.A.C. method, *Methods of Analysis*, 550, 19(b) salol.

Acetophenetidin and acetylsalicylic acid.—Wash the beaker and filter containing the acetophenetidin and acetylsalicylic acid with $CHCl_3$, receiving the filtrate in a separator until extraction is complete (usually 50–75 ml. of $CHCl_3$ is sufficient). Shake the $CHCl_3$ solution with 15 ml. of the $NaHCO_3$ solution. Withdraw the $CHCl_3$ into a second separator, wash with 5 ml. of the $NaHCO_3$ solution, and filter through a funnel containing a pledget of cotton moistened with $CHCl_3$. Add about 20 ml. of $CHCl_3$ to the separator containing the 15 ml. of $NaHCO_3$ solution, shake, and withdraw into the second separator. Wash through the second separator and filter the $CHCl_3$ through the same filter as before. Repeat the process once more with about 15 ml. of $CHCl_3$. Evaporate the combined $CHCl_3$ solutions to dryness on the steam bath, dry in an oven at 100° C., and weigh as acetophenetidin. Combine the $NaHCO_3$ solutions into one separator, acidify with HCl (1+1), and extract the acetylsalicylic acid with successive portions of $CHCl_3$, etc., as directed in Method I.

COLLABORATIVE WORK

Two samples were sent out for collaborative work. Sample A was a mixture prepared by the associate referee, containing $33\frac{1}{3}$ per cent each of acetylsalicylic acid, acetophenetidin, and salol.

TABLE 2.—*Collaborative results with Method II (per cent)*

Sample A

COLLABORATOR	ACETYLSALICYLIC ACID		ACETOPHENETIDIN		SALOL	
	FOUND	AVERAGE RECOVERY	FOUND	AVERAGE RECOVERY	FOUND	AVERAGE RECOVERY
H. H. Shull	32.85		33.90		32.61	
	32.30	97.7	33.22	100.7	33.32	98.9
F. H. Hedger	32.10	96.3	33.60	100.8	34.30	102.9
O. C. Kenworthy	32.77		33.49		33.52	
	32.33	97.7	33.67	100.8	32.52	99.4
D. C. Grove	32.33		33.23		33.40	
	33.17	98.1	33.61	100.3	33.03	99.7
C. F. Bruening	31.95		33.18		34.10	
	32.10	96.1	32.67	98.8	34.25	102.6
Average	32.43	97.3	33.40	100.2	33.45	100.4

	Sample B					
H. H. Shull	29.28 28.45	99.6	28.50 28.92	99.0	29.96 29.55	102.6
F. H. Hedger	26.70	92.1	30.90	106.6	28.30	97.6
O. C. Kenworthy	27.96 28.15	96.8	28.52 28.39	98.1	29.75 29.77	102.6
D. C. Grove	28.88 28.55	99.0	29.23 29.05	100.5	28.89 29.62	100.9
C. F. Bruening	27.75 27.62	95.5	28.55 28.80	98.9	30.24 30.24	104.3
Average	28.15	97.1	28.98	99.9	29.59	102.0

Sample B was a mixture prepared by the associate referee, containing acetylsalicylic acid, 29.0 per cent; acetophenetidin, 29.0 per cent; salol, 29.0 per cent; starch, 11.6 per cent, and talc, 1.4 per cent.

COMMENTS OF COLLABORATORS

H. H. Shull.—Method I is preferred for the separation of the aspirin, since the temperature can be more readily controlled. With Method II acetophenetidin is lost, since it is soluble in petroleum benzin (0.015 gram in 100 ml. approx.). For control work, Method I should be used for aspirin and acetophenetidin and salol should be determined on a separate weighing with Method II.

F. H. Hedger.—In carrying out the procedures outlined in the two methods, we experienced somewhat greater inconvenience with the petroleum benzin separation described in Method II than with the corresponding $CHCl_3$ separation of Method I. Considering the numerous transfer operations of both methods, it seems rather difficult to obtain results agreeing to within less than 1%.

O. C. Kenworthy.—Both methods are quite workable. In Method I, you did not mention washing the $CHCl_3$ extractions from the $NaHCO_3$ solution. I did this and believe such a step should be included in the directions. Method I seemingly gives better results and is, I believe, the better method. Dry extractions always introduce a possibility of error through creeping, dissolving small amounts of the insoluble portions, etc.

R. L. Herd.—I believe that Method I is quite satisfactory. It might be wise to add some cracked ice to the separator during the separation of the acetophenetidin and salol from the acetylsalicylic acid. Also, the separation of the acetophenetidin and the salol from the acetylsalicylic acid might be speeded up by combining the first four $CHCl_3$ extracts in the second separator before washing with $NaHCO_3$.

CONCLUSIONS

It is believed that Method I is the more satisfactory. The results with Method II, while not so bad as an average, do not seem to agree well on duplicate determinations. As some of the acetophenetidin dissolves in petroleum benzin, a low result should be obtained for this ingredient;

however, slightly high results were obtained. This is no doubt due to an incomplete extraction of the salol by the volume of petroleum benzin used.

The agreement among collaborators is believed to be as satisfactory for Method I as could be expected in a mixture of this type, where all three ingredients are determined quantitatively on a single sample.

It is recommended that Method I, proposed for the determination of acetylsalicylic acid, acetophenetidin and salol, be adopted as tentative; and that the subject be closed.

No report on aminopyrine and phenobarbital in mixtures was given by the associate referee.

REPORT ON ELIXIR OF TERPIN HYDRATE AND CODEINE

By JONAS CAROL (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

For two years collaborative studies of methods of analysis of elixir of terpin hydrate and codeine have been made, *This Journal*, **21**, 575 (1938); **22**, 736 (1939). The results, while generally good, did not in all cases prove to be satisfactory. The terpin hydrate results tended to be too high, and the codeine results too low. These inaccuracies seemed to arise from two causes: (1) The lack of a method of drying the terpin hydrate residue that would be unaffected by humidity, and (2) the small (3 ml.) titration for codeine.

This year an attempt was made to remedy these two faults. The codeine and terpin hydrate determinations were made on separate portions of sample. This was necessary because the large sample taken for the codeine determination contained more terpin hydrate than could be extracted with a reasonable number of extractions. The 25 ml. sample used contained codeine equivalent to approximately 8.0 ml. of 0.02 *N* acid. This was considered a reasonably large titration.

A method of evaporating and drying the terpin hydrate extract in a current of dry air, as suggested by H. H. Shull, private communication, was incorporated in the method. A calcium chloride drying train used first to dry the air proved inadequate in damp weather. A sulfuric acid drying train proved quite satisfactory.

Samples of elixir of terpin hydrate and codeine NF IV, 100 ml., containing 1.7000 grams of terpin hydrate and 0.2000 gram of codeine and water were submitted to the collaborators to be analyzed.

Table 1 contains the results obtained by the collaborators and the associate referee.

TABLE 1.—*Recovery of terpin hydrate and codeine*

COLLABORATOR	TERPIN HYDRATE		CODEINE	
	G/100 ML.	PER CENT	G/100 ML.	PER CENT
C. B. Stone	1.719	101.1	0.201	100.5
St. Louis	1.708	100.5	0.199	99.5
M. M. Spruiell	1.690	99.4	0.198	99.0
Cincinnati	1.678	98.7	0.199	99.5
			0.197	98.5
R. Hyatt	1.702	100.1	0.204	102.0
Cincinnati	1.710	100.6	0.205	102.5
H. H. Shull	1.702	100.1	0.195	97.5
Philadelphia	1.706	100.4	0.193	96.5
H. R. Bond	1.680	98.8		
Kansas City	1.680	98.8		
J. Carol	1.697	99.8	0.201	100.5
Chicago	1.702	100.1	0.201	100.5

DISCUSSION

The results obtained from all the collaborators were considered quite satisfactory for a product of this type. The method overcomes the two objections of the former methods, the small titer for codeine, and the uncertainty of drying the terpin hydrate residue.

It is recommended that the method be adopted as a tentative method.

The method was adopted as tentative and is published in *Methods of Analysis, A.O.A.C.*, 1940, 579.

REPORT ON YELLOW MERCURIC OXIDE OINTMENT

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

The work undertaken this year continued along the lines previously reported by Associate Referee T. F. Pappe, *This Journal*, **15**, 409 (1932), which included collaborative results on two methods. The Association recommended further study of one of these, the Rupp iodine titration method, but apparently this method has not been applied to this product. In the meantime a mercuric sulfide method embodying the principles outlined by Pappe has been included in U.S.P. XI.

The time available was devoted mainly to preparing and testing a batch of strictly U.S.P. ointment of known mercuric oxide content for subsequent control assays. The purity of the mercuric oxide used was deter-

mined; the ointment base was tested; and assayable size batches of ointment were prepared according to the U.S.P. so that the whole amount of each was used for a determination. These were then assayed by the U.S.P. directions to determine what details of manipulation would produce uniform results and effect complete recovery. Following the technic thus indicated, the uniformity as well as total mercury oxide content of the large batch was checked by multiple determinations of portions taken from different parts of the container.

Preliminary trials of manipulative technic included (1) unsuccessful attempts to transfer the ointment from a beaker by warming and washing with ether, (2) unfortunate experiences in dissolving the base in ether, and (3) loss of determinations because mercuric oxide crept into the channel of the stopcock and out of contact with the acid. A tendency toward emulsions (poor separation of water layer) due to diminution of ether layer was evident.

The method used follows:

Weigh the sample on glassine paper about 2 inches square, roll into a pellet, and insert into a horizontally placed separator near the top stopper. Immerse side of separator held in horizontal position in water bath at 60°–80° C. until a portion of the ointment flows. Cool to cause the entire sample to adhere to the side near the top of separator, add about 75 ml. of ether, and warm again in the horizontal position, holding the top stopper loosely in the opening until the ether boils gently. Shake vigorously 5–10 seconds, release the pressure, and repeat the warming and shaking until the mixture is uniformly cloudy and not lumpy. Add the acid immediately. Shake the acid-ether mixture 3 times per second for 2 minutes. Wash with 10 ml. portions of distilled water 8 or 10 times or until the turbidity test with $AgNO_3$ shows less than that caused by 0.15 mg. of $HgCl_2$. If a second portion of acid is used to hasten or complete the extraction make 4 water washings after the first portion of acid and at least 4 after the second. Pass in H_2S until the supernatant liquid is clear. Directions under the $HgCl_2$ assay, U.S.P. XI slightly modified, were followed in washing the HgS to remove sulfur.

Since the sulfide method was used for the ointment assays, it was likewise used to determine the purity of the mercuric oxide control chemical. The results are given in Table 1. Since this method showed an average recovery of 100.3 per cent, all weights of mercuric oxide taken for assays were multiplied by 1.003. Therefore the per cent recoveries represent the effect of the mixed ointment constituents. The mercuric oxide was also assayed by thiocyanate titration, and the results showed an average of 100.05 per cent by two determinations. Drying at 110.° C., specified by the U.S.P., showed average loss of 0.11 per cent. Adding this loss to the 0.10 per cent non-volatile impurities declared gave an indicated purity of 99.79 per cent, or about 0.5 per cent less than that shown by the sulfide method. The ointment base was found to contain sulfide precipitable materials equivalent to 0.7 per cent mercuric oxide, as shown in Table 1.

These details were followed in making the assays reported in Table 2.

TABLE 1.—*Purity of HgO and blank on ointment base by sulfide method*

	WEIGHT TAKEN	WEIGHT FOUND	HgO
	gram	*gram*	*per cent*
	0.1425	0.1429	100.3
	0.1425	0.1426	100.1
	0.3345	0.3361	100.5
	0.3603	0.3617	100.3
Average			100.3
Ointment Base	10.00	0.0008, 0.0007 Av.	.7*

* This excess was not deducted from the results in Table 2.

TABLE 2.—*HgO found by modified U.S.P. XI method in prepared ointments*

PRODUCT	WEIGHT TAKEN	WEIGHT RECOVERED	RECOVERY 1 PORTION HCl	RECOVERY 2 PORTIONS HCl*
	gram	*gram*	*per cent*	*per cent*
Individual Batches				
No.				
5	0.1068	0.1062	99.4	—
6	0.1027	0.1019	99.2	—
6(a)	0.1099	0.1099	100.0	—
7	0.1089	0.1081	99.3	101.1
8	0.1107	0.1093	98.7	100.2
9	0.1062	0.1048	98.7	100.8
10	0.1072	0.1060	98.9	100.7
Average			99.3	100.7
Large Batch‡				
Determination				
C1	0.1032	0.1024	99.2	100.6
C2	0.1032	0.1026	99.4	100.0
C3	0.1032	0.1027	99.5†	100.7
C3(a)	0.1032	0.1042	—	101.0
C4	0.1032	0.1026	99.4	99.5
C5	0.1032	0.1041	—	100.9
C6	0.1032	0.1038	100.6	101.4
C7	0.1032	0.1035	100.3	100.9
Average			99.7	100.6

* To satisfy a curiosity as to the quantity of Hg not extracted by 1 portion of acid and 8 or 10 portions of water, the ether solution was again extracted with HCl followed by several portions of water; this extract was carried through precipitation and weighed; and the results were added to those previously obtained.

† The precipitate in the crucible was refluxed with hot ether and reweighed. The loss, 0.0012 gram, is believed to be the ointment base not removed by cold solvents. All other crucibles used for determinations C1 to C7 were then refluxed with hot ether without appreciable change. Refluxing with hot CCl_4 also produced no change. They were then digested overnight in cold CCl_4 without change.

‡ Contained 7.047 grams, or 1.028 per cent HgO in 681.25 grams of ointment.

DISCUSSION OF RESULTS

In the assays of "Individual Batches" when one portion of hydrochloric acid was used, the recoveries (Table 2) vary from 98.7 per cent to 100.0

per cent and average 99.3 per cent, which indicates reasonably uniform results. The results on the "Large Batch" range from 99.2 per cent to 100.6 per cent, which indicates uniformity of the batch.

Average recoveries of 100.7 per cent and 100.6 per cent, respectively, are shown on the "Individual" and the "Large" batches when two acid extractions were made instead of one. That these results are closer to the correct values can be seen when the sulfide precipitate amounting to the equivalent of 0.7 per cent of mercuric oxide in the blank on the ointment base is deducted from the above. This leaves practically 100 per cent average recovery. This is 1–1.4 per cent higher than when extraction is made with one portion of acid.

SUMMARY

A batch of U.S.P. Yellow Mercuric Oxide Ointment of sufficient size for limited investigational work was prepared and found to be uniform. It is available for the next associate referee on this subject if desired.

It is recommended that in view of the possible advantages of a volumetric method the Rupp iodine titration method be studied in regard to its applicability to this product.

No report on rhubarb and rhaponticum was given by the associate referee.

REPORT ON THEOPHYLLINE SODIUM SALICYLATE

By M. HARRIS (U. S. Food and Drug Administration, New Orleans, La.), *Associate Referee*

The work on this topic was continued in accordance with the recommendation of Subcommittee B. Since application of the proposed methods had failed to yield uniform results in the determination of the commercial product in the presence of excipients, *This Journal*, **21**, 587 (1938), it seemed desirable to investigate first the effectiveness of the proposed methods in assaying the product itself. Accordingly, a commercial specimen was examined by the gravimetric and volumetric methods that previously in collaborative study had yielded concordant results when applied to a synthetic mixture of three parts of Theophylline, U.S.P. and four parts of U.S.P. Sodium Salicylate.

Results of Analysis

ASSAY	THEOPHYLLINE-SODIUM *per cent*	SODIUM SALICYLATE *per cent*
Gravimetric	47.59–47.31	48.65–48.81
Volumetric	47.77–47.53	48.51–48.67

It was also determined that the specimen contained 0.7 per cent moisture and 0.15 per cent caffeine.

If the respective components in the product ($NaC_7H_7N_4O_2$) described in Merck's Index, 4th Ed. p. 519, as $NaC_7H_7N_4O_2 \cdot C_6H_4 \cdot (OH) \cdot COONa$ are chemically combined, then the product theoretically should contain 55.81 per cent theophylline sodium and 44.19 per cent sodium salicylate. However, from the analysis by the above procedures, where the components are separated alternately in the two methods, it is apparent that the respective ingredients are present as a mixture and in practically equal proportions.

A mixture of five parts of the commercial specimen, one part of starch, and one part of lactose was then examined by Methods 1 and 2, *This Journal*, 21, 590 (1938), Method 2 being modified by repeating the extractions with chloroform-ethyl alcohol solvent until both the alkaloid and salicylic acid are removed completely, and the substitution of one 10 ml. portion of water acidified with several drops of 1 and 3 hydrochloric acid, for the 2–10 ml. portions of water in washing the combined extracts.

Results of Analysis

ASSAY	THEOPHYLLINE SODIUM *per cent*	SODIUM SALICYLATE *per cent*
Gravimetric	47.44	48.61
Volumetric	47.36	48.89

In view of the results obtained, the Associate Referee recommends that further study be made of the determination of the entire and exact composition of the commercial product and that the modified methods of analysis in presence of excipients be submitted to collaborative study.

No report on mandelic acid in mixtures was given by the associate referee.

REPORT ON PHYSOSTIGMINE SALICYLATE

By GEORGE M. JOHNSON (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

Physostigmine salicylate is official in U.S.P. XI, but no assay is provided. The several published methods for the assay are essentially the same and conform to the general scheme of alkaloidal assays. Physostigmine is a strongly basic alkaloid. Its aqueous solutions turn red on exposure to air and light and become darker with the passage of time, owing to the formation of the red coloring matter rubreserine. This change is facilitated by the presence of alkalies. Ammonia with the aid of heat changes the alkaloid into a blue residue. It is because of this sensitivity to

alkalies and ammonia that the methods substitute either sodium carbonate or bicarbonate. The Pharmaceutical Standards[1] specify the latter.

The method given here is essentially the one appearing in the 1935 edition of the Pharmaceutical Standards except that chloroform is substituted for ether as the immiscible solvent. This method was sent to the collaborators, together with a mixture of lactose and physostigmine salicylate. The alkaloidal salt amounted to 2.44 per cent of the mixture and was of U.S.P. XI grade. It was supplied by a reputable manufacturer. The method is as follows:

METHOD

PREPARATION OF SAMPLE

Count and weigh a representative number of tablets, calculate the average weight, and powder in a mortar.

DETERMINATION

Weigh sufficient powdered material to contain about 0.065 gram of physostigmine salicylate. Transfer to a separator and dissolve in water. Make alkaline with solid $NaHCO_3$ and extract at once with $CHCl_3$, using 30, 20, 20, 20, and 10 ml. portions. Wash each extract with 5 ml. of water and filter into a beaker through cotton moistened with $CHCl_3$. Test for complete extraction, using a 5 ml. portion of $CHCl_3$. Evaporate the combined extracts to about 5 ml. on water bath, using a blast of air. Complete the evaporation without the aid of heat. Dissolve the residue in a few ml. of neutral alcohol. Add an excess of 0.02 *N* H_2SO_4 and titrate the excess acid with 0.02 *N* NaOH, using methyl red indicator. 1 ml. of 0.02 *N* $H_2SO_4 = 0.00826$ gram of physostigmine salicylate.

The collaborative results are shown in the table.

COLLABORATOR	PHYSOSTIGMINE SALICYLATE *per cent*	RECOVERY *per cent*
H. R. Bond	2.29	93.9
Kansas City	2.29	93.9
H. W. Conroy	2.34	95.9
Kansas City	2.33	95.5
R. Hyatt	2.29	93.9
Cincinnati	2.34	95.9
	2.35	96.3
I. S. Shupe	2.37	97.1
Baltimore	2.37	97.1
G. M. Johnson	2.40	98.8
	2.41	98.4
Average	2.34	96.1

The collaborators report no difficulty with the assay and yet the results show incomplete recovery of the alkaloid. The Associate Referee cannot point out any particular reason for this discrepancy. The method fails

[1] Pharmaceutical Standards Including Tolerances and Methods of Analysis, 1935.

to mention the indicator to be used. Methyl red should have been specified and was used by all the collaborators.

RECOMMENDATIONS

It is recommended—

(1) That the method of assay for physostigmine salicylate be studied further and that more collaborative work be done.

(2) That the assay of physostigmine salicylate be extended to ointments and other mixtures that occur commercially.

No report on the separation of acetanilid and salol was given by the associate referee.

REPORT ON ARECOLINE HYDROBROMIDE

By HENRY R. BOND (U. S. Food and Drug Administration, Kansas City, Mo.), *Associate Referee*

Since no definite chemical assay method for arecoline hydrobromide has been established, it was deemed advisable to devise a method that would be reasonably specific. A search of available literature produced only enough information to provide a nucleus for preliminary investigation.

After a study of the physical and chemical natures of arecoline alkaloid and the hydrobromide, the Associate Referee considered the three following possibilities of assay worthy of trial:

(1) Steam distillation, because arecoline is volatile with steam, and subsequent titration of the alkaloid.

(2) Extraction from alkaline solution with a volatile solvent with titration of the extracted alkaloid.

(3) Precipitation with silico-tungstic acid in a manner similar to the official method of assay for nicotine.

A limited amount of experimental work was done with assay possibilities (1) and (2). The results obtained with the second method indicate the suitability of that type of assay for the product. The amount of work done on the first method, while incomplete, encouraged the Associate Referee to believe that further development will produce a usable assay. No work was done with the third method, but if distillation by steam is found to be suitable, it is believed that this form of assay may be used.

It is recommended that the topic be studied further.

REPORT ON BENZEDRINE

By JAMES H. CANNON (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

In accordance with a recommendation made last year, study of methods for the determination of benzedrine was begun. This compound, which is

widely used as an inhalant, is mentioned in the literature under a variety of names. According to New and Non-Official Remedies, 1937, it is a synthetically prepared racemic mixture of bases having the formula $C_6H_5CH_2CHNH_2CH_3$ and is described as racemic benzyl-methyl carbinamine, or racemic desoxynor-ephedrine. In 1938 a decision was announced by the Council on Pharmacy and Chemistry of the American Medical Association that "the non-proprietary name 'Amphetamine' shall be used for this drug." The council further submits, as a descriptive chemical name, α-methyl phenethylamine. The compound is described in New and Non-Official Remedies as a colorless mobile liquid, b.p. 200°–203° C. (with slight decomposition); specific gravity at 25° C., 0.931; odor, strongly basic; taste, burning; soluble in ether and alcohol and slightly soluble in water.

The method that first suggests itself is that described in New and Non-Official Remedies, namely, steam distillation of the base into standard acid solution and back titration with standard sodium hydroxide solution. Preliminary determinations made on compressed tablets labeled to contain 10 mg. of benzedrine sulfate per tablet yielded low results; that is, less than 10 mg. per tablet. These figures, however, have little if any value since the amount of benzedrine sulfate actually present was not known with certainty. An effort was made to obtain a supply of the pure compound free from excipients, but owing to certain misunderstandings this was not done in time to permit any collaborative work. It is believed now that authentic material will be available for the work next year, and it is the expectation of the Associate Referee that collaborative study may be made then. Accordingly, the Associate Referee recommends that study of methods for the determination of amphetamine (benzedrine) in inhalants be continued during the coming year.

Only a verbal report of progress on the subject of plasmochine was given by the associate referee.

No report on hydroxyquinoline sulfate was given by the associate referee.

No report on pepsin was given by the associate referee.

No report on ipecac and opium powder (Dover's powder) was given by the associate referee.

REPORT ON NICOTINIC ACID

By P. S. JORGENSEN (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

Nicotinic acid is a substance that has recently gained considerable

recognition as a therapeutic agent because of widespread reports in medical literature concerning its curative effect on some of the symptoms of pellagra.

Chemically nicotinic acid is beta-pyridine carboxylic acid, $C_5H_4 \cdot N(1) : COOH(3)$. It occurs as white odorless crystals or powder. It is slightly soluble in cold water, practically insoluble in ether and chloroform, but soluble in hot water and alcohol. The melting point is 235° C.[1]

Because of its insolubility in immiscible solvents the usual methods of separation could not be used. Beilstein[2] states that it sublimes undecomposed. This suggested a means by which it might be separated from substances with which it is ordinarily combined in tablets and ampuls. These substances are starch, lactose, and talc in tablets and sodium chloride in ampuls. Experiments were made with these mixtures as well as pure nicotinic acid under varying sublimation procedures with promising results. Finally a method was developed and applied to accurately prepared samples. Excellent recovery was obtained. A tablet mixture was prepared consisting of lactose, 3.0 grams; starch, 3.0 grams; talc 2.5 grams; and nicotinic acid, 1.5 grams (15 per cent nicotinic acid), all dried at 100° C. A solution of nicotinic acid in physiological salt was made so that each 5 ml. of solution contained 0.030 gram of nicotinic acid (0.6 per cent). Samples of these two preparations were sent to several collaborators to be analyzed according to the method formulated, which was adopted as tentative and published in *Methods of Analysis, A.O.A.C.*, 1940.

The results obtained are shown in the table:

COLLABORATOR	TABLET MIXTURE NICOTINIC ACID 15%		AMPUL SOLUTION NICOTINIC ACID 0.6%	
	per cent	*M.P.*	*per cent*	*M.P.*
M. L. Yakowitz	14.9	230	0.598	230
Food & Drug Adm.	14.8		0.594	
San Francisco				
R. K. Snyder	13.1	238	0.57	235
A. Ph. A. Laboratory	14.0		0.60	
Washington				
Norman E. Foster	13.96	233	0.582	233
Food & Drug Adm.	14.19		0.578	
Philadelphia				
Harley G. Underwood	14.9	230	0.60	230
Food & Drug Adm.	14.9		0.60	
Chicago				
P. S. Jorgensen	14.88	231	0.60	231
Food & Drug Adm.	14.97		0.598	
San Francisco				

[1] *J. Am. Med. Assoc.*, 111, 27 (1938).
[2] *Organische Chemie*, 3rd Ed., Vol. 4, 143–44.

COMMENTS BY COLLABORATORS

Harley G. Underwood.—While the sublimate appears to be even and fairly adherent, I found it somewhat difficult to remove the suction flask without shaking off a small quantity of nicotinic acid. In such cases a second sublimation was made and combined with the first.

The assay for nicotinic acid in a recently proposed U.S.P. supplement is by titration with 0.1 *N* NaOH in aqueous solution, and with phenolphthalein as indicator. Perhaps it would be better to titrate the sublimate with 0.02 *N* NaOH and to use recently boiled distilled water. Titrating one of the sublimates of the liquid preparation I obtained 0.594 gram per 100 ml. as compared with 0.60 gram per 100 ml. gravimetrically.

Norman E. Foster.—The only difficulty experienced was in accurately weighing the sublimate, due to some humid weather at the time. As this appears to be the most critical point in the assay I would suggest that more explicit directions for weighing be included in the method, namely the use of a tared beaker, and a definite cooling time in the desiccator (one hour suggested). The weighing of the empty beaker should be made after the same time of heating and cooling.

In my opinion a larger sample is indicated.

SUMMARY

Results obtained indicate that this method gives satisfactory results for nicotinic acid when combined with the usual diluents in tablets and ampuls. Due to the limitations of the apparatus small quantities must be used, necessitating extreme accuracy in weighing. The sample should be adjusted to yield not over 0.040 gram of sublimate. Larger quantities are too easily shaken off when the condenser tube is removed. It seems more desirable to weigh the sublimate in order that it may be used for identification.

It is recommended[3] that the subject be studied further with a view to improvement.

REPORT ON EPHEDRINE IN JELLIES

By E. H. GRANT (U. S. Food and Drug Administration, Boston, Mass.), *Associate Referee*

When notified of his assignment, the Associate Referee wrote to various laboratories to ascertain whether difficulties had been encountered in the determination of ephedrine salts in jellies that were different from the difficulties encountered in assaying other ephedrine preparations. With one exception all of the replies were negative.

The sample giving difficulty was examined by the Associate Referee, who submitted some detailed directions to the original chemist, who then reexamined the sample. Both chemists obtained satisfactory results.

[3] For report of Subcommittee B and action by the Association, see *This Journal*, 23, 58 (1940).

It appears that the difficulties were due to a misinterpretation of the A.O.A.C. directions concerning "moderate heat." The Associate Referee has found that in the assay of ephedrine preparations it is not advisable, in evaporating the ether, to allow the beaker to get appreciably warmer than room temperature. This has been the experience of other chemists consulted.

In reporting the method for ephedrine originally, Glycart and Paul, *This Journal*, **13**, 329 (1930), showed that losses occur if too much heat is used, and also that good results are obtained if "moderate" heat is used for the first portion of the evaporation. It is advisable to define "moderate" definitely enough to avoid overheating.

A sample of jelly of ephedrine sulfate was made according to the formula in National Formulary VI and assayed by the A.O.A.C. method. Satisfactory results were obtained. No samples were submitted to collaborators. There appears to be no reason for adopting an A.O.A.C. method for the assay of ephedrine in jellies differing from the method used for other preparations of this alkaloid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the official method for the assay of ephedrine in inhalants, *Methods of Analysis, A.O.A.C.*, 1935, 557, be amended by adding, after the words "by the aid of a current of air," these words: "so adjusted that the temperature of the ether will not rise appreciably above room temperature, nor fall enough below such temperature as to cause any appreciable condensation of water."

(2) That the study of this subject be discontinued.

REPORT ON CAFFEINE

By JOHN R. MATCHETT (U. S. Bureau of Narcotics, Washington, D. C.), *Associate Referee*

In view of certain criticisms of that part of the method for caffeine determination that has to do with purifying contaminated caffeine recovered from mixtures, *Methods of Analysis, A.O.A.C.*, 1935, 544, the subject was opened for collaborative study.

The success of the method in removing contaminating material will be conditioned, of course, by the nature of the impurities. Hence, the problem appears to resolve itself into a determination of whether caffeine can be quantitatively recovered by precipitation with Wagner's reagent from aqueous solution under the specified conditions.

Preliminary results obtained in this laboratory indicated that under

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **23**, 58 (1940).

these conditions considerable loss of caffeine occurred even when the precipitation and standing were carried out at refrigerator temperature (8° C.).

A sample was prepared by dissolving 20.00 grams of pure caffeine in sufficient 0.2 *N* sulfuric acid solution to make 1000 ml. The solution was divided into ten equal parts and submitted to collaborators for analysis. It was requested that determinations be made by simple extraction as well as by precipitation, both at room temperature and at refrigerator temperature. Tables 1 and 2 record the data and illustrate the unsatisfactory recoveries obtainable. Percentage recoveries are calculated on the basis of results obtained by each collaborator on direct extraction.

TABLE 1.—*Recovery of caffeine obtained at room temperature with the present official method*

COLLABORATOR	SAMPLE	TIME OF STANDING	BY DIRECT EXTRACTION	BY METHOD	RECOVERY
	ml.	*hours*	*gram*	*gram*	*per cent*
Shupe	5		0.1003		
	5	1		0.0831	82.8
Glycart	10		0.2002		
	5	1		0.0815	81.5
Bond	10		0.2000		
	5	1		0.0794	79.4
Foster	10	1		0.1665	83.2
	10	overnight		0.1690	84.5
Fulton	10		0.1999		
	10			0.1714	85.7
Jorgensen	10		0.2026		
	10	1		0.1705	84.1
	10	1		0.1710	84.4
This	10		0.2009		
Laboratory	10	1		0.1772	88.2
	10	1		0.1631	81.2

During the course of collaborative work on the method C. C. Fulton, of the Bureau of Internal Revenue Laboratory, St. Paul, Minn., suggested that excellent recoveries of caffeine might be had if the solution were further acidified at the time of precipitation.

The method with the change suggested was adopted as official and published in *Methods of Analysis, A.O.A.C.*, 1940.

Requests were immediately made of collaborators to insert the change

in the method and report results obtained. Table 3 records the excellent recoveries made by all analysts.

TABLE 2.—*Recovery of caffeine at refrigerator temperature with present official method*

COLLABORATOR	SAMPLE	TIME STANDING	DIRECT EXTRACTION	RECOVERY	
	ml.	*hours*	*gram*	*gram*	*per cent*
Shupe	5		0.1003		
	5	1		0.0914	91.1
Stewart	10		0.2025		
		24		0.1908	94.2
		24		0.1892	93.4
Glycart	10		0.2002		
	10	1		0.1752	87.5
	5	1		0.0878	87.7
Bond	10		0.2000		
	10	1		0.1801	90.0
	5	1		0.0896	89.6
Foster	10	1		0.1822	91.1
	10	4		0.1806	90.3
	10	overnight		0.1803	90.1
Fulton	10		0.1999		
	10	1		0.1825	91.3
	10	1		0.1818	90.9
Jorgensen	10		0.2026		
	10	1		0.1857	91.6
	10	overnight		0.1835	90.6
Reindollar	5		0.1035		
	10		0.2035		
	5	1		0.0905	88.2
	10	1		0.1620	78.9
This	10		0.2024		
Laboratory	10	1		0.1967	97.2
	10	1		0.1965	97.1
	10	1		0.1955	96.6

In order to test the efficacy of the method in removing colored vegetable substances from impure recovered caffeine, a few analyses were made of 10 ml. samples of a solution similar to the standard one to which had been added certain plant extractives. The caffeine was extracted from these,

and the highly colored recovered material was treated by the modified procedure. The caffeine finally obtained was much whiter than the material first extracted, but it was found, as expected, that certain plant

TABLE 3.—*Recovery of caffeine obtained with proposed method*

COLLABORATOR	SAMPLE	TIME STANDING	TEMPERATURE	BY DIRECT EXTRACTION	BY METHOD	RECOVERY
	ml.	*hours*		*gram*	*gram*	*per cent*
Shupe	5			0.1003		
	5	1	Refrigerator		0.0998	99.5
	5	1	Refrigerator		0.0997	99.4
Glycart*	10			0.2014		
	10	1	Refrigerator		0.2008	99.7
	10	1	Refrigerator		0.2004	99.5
Berry*	10			0.2030		
	10	1	Refrigerator		0.2020	99.5
Stewart	10			0.2009		
	10	1	Room		0.2019	100.5
	10	1	Refrigerator		0.1999	99.5
	10	1	Refrigerator		0.1999	99.5
Foster*	10			0.2008		
	10	1	Room		0.2011	100.1
	10	1	Refrigerator		0.2013	100.2
Fulton	10			0.1999		
		1	Refrigerator		0.2004	100.2
Reindollar	5			0.1035		
	10			0.2035		
	5	1	Refrigerator		0.1015	98.9
	10	1	Refrigerator		0.2020	98.4
Valaer	20		Refrigerator		0.4004	100.1
This	10			0.2009		
Laboratory	10	1	Refrigerator		0.2002	99.7
	10	1	Refrigerator		0.2009	100.0
	10	1	Room		0.2000	99.5
	10	1	Refrigerator		0.2001	99.6
	10	1	Refrigerator		0.1992	99.1

* Caffeine sample for analysis prepared by collaborator.

extractives are not completely eliminated. The results are recorded in Table 4.

TABLE 4.—*Caffeine recovered from plant extractives*

SUBSTANCE ADDED	CAFFEINE RECOVERED	
	gram	*per cent*
None	0.1994	—
1 *ml.* F.E. Gentian	0.2003	100.4
1 *ml.* F.E. Ginger	0.2050	102.8
1 *ml.* Sirup Squill	0.2118	106.2
2 *ml.* F.E. Celery Seed	0.2113	106.0

COMMENT BY COLLABORATORS

All collaborators agreed that quantitative results could not be obtained by the present method, but could be obtained by the proposed method. No suggestions were received for improving the method in regard to precipitation and recovery. It was suggested, however, that the drying of caffeine in the air may not be wholly satisfactory, especially in very humid weather. In this laboratory it was found that if the chloroform were driven off on the steam bath, before a fan, appreciable changes in weight of the beaker and caffeine were seldom found.

DISCUSSION

Failure to obtain satisfactory recoveries by the present method indicates that the difficulties encountered are due to failure of Wagner's reagent to quantitatively precipitate caffeine from aqueous solution under the conditions specified. The method now proposed does accomplish this and should, accordingly, prove satisfactory for the purpose intended.

It is apparent that some simplification of the method might be effected by dissolving the contaminated caffeine in an acid solution sufficiently concentrated so that further acidification at the time of precipitation would be unnecessary. The results indicate also that when the new procedure is used good recoveries may be expected when analysis is carried out at room temperature. Since these modifications have not been extensively studied the wording of the present method has not been changed except for the specification of adding additional acid.

The presence of the filter paper in the separatory funnel makes the final caffeine extraction very difficult from the manipulative standpoint. At this Laboratory, use of a G4 fritted glass filter has been found to give equally good results, the caffeine periodide being simply washed off by sulfite solution. It was found that a G3 filter does not retain the precipitate.

It is recommended that the method, modified as proposed herein, be adopted as tentative.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Joseph Levine and Louis Ben-

jamin, of this laboratory, for their able assistance in carrying out this investigation; to the collaborators for their splendid cooperation; and, especially, to C. C. Fulton, whose suggestion makes it possible to recommend retention of the method.

ASH DETERMINATION IN FOODS WITH AN ALKALINE BALANCE

In the paper on this subject, *This Journal*, 23, 680 (1940), the names assigned to the various food products investigated were chosen as being simply descriptive of their general characteristics. For example, the samples called preserves were not prepared to conform to any requirements for composition as set forth in identity standards.—H. J. WICHMANN.

CONTRIBUTED PAPERS

ASSAY OF OINTMENT OF RED MERCURIC IODIDE*

By RUPERT HYATT (U. S. Food and Drug Administration, Cincinnati, Ohio)

The British Pharmaceutical Codex describes the preparation of ointment of red mercuric iodide as—

	METRIC	IMPERIAL
Red mercuric iodide, finely sifted	4 g.	70 grs.
Benzoinated lard	96 g.	3 oz. 367½ grs.
Mix		

Several methods of analysis are mentioned in the literature, of which Adam's[1] is the simplest. The procedure involves only the dissolving of the base in benzene, decanting, and weighing the residue. An alternative method consists of shaking out the benzene solutions with potassium iodide solution and determining the mercury as mercuric sulfide.

Mitchell[2] reports successful application of the Evans method.[3] This involves shaking the ointment in a separator with hot, mixed acids (HNO_3-1, HCl-1, water-2), neutralizing, precipitating with hydrogen sulfide, dissolving the mercuric sulfide, and percolating with copper. A caution was given against dissolving red mercuric iodide in potassium iodide solution and precipitating with hydrogen sulfide since "it seems impossible to wash all the iodide out of the sulfide precipitate." Mitchell's method seemed impracticable and was not used.

The British Pharmacopoeia does not list the ointment but assays mercuric iodide on the basis of its iodide content. The mercury content was desired since the composition may not be exactly that indicated by the formula. In addition, other halides and the ointment base would interfere.

The proposed method for citrine ointment[4] was found inapplicable. In the titration with NH_4SCN a red precipitate, perhaps red mercuric iodide, was formed and obscured the end point. Precipitation with hydrogen sulfide from a potassium iodide solution gave results of 105–106 per cent of theoretical. The precipitate was well washed with carbon tetrachloride, which should eliminate any sulfur contamination. It is possible that the high results may be due to incomplete conversion to the sulfide or to occlusion of the red mercuric iodide. It appears necessary to remove iodides.

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 30, 31, and November 1, 1939.

[1] *J. Pharm. Chem.*, [6], 30, 300 (1909).

[2] *Analyst*, 51, 293 (1926).

[3] *C. A.* 20, 2631 (1926).

[4] H. O. Moraw, Unpub. Work, U. S. Food and Drug. Adm., Chicago, Ill.

Assays of an ointment sample made up to be 4 per cent were made by the use of monoethanolamine.[5] Three determinations gave 3.74, 3.88, and 3.71 per cent. Results were consistently low, so this method was abandoned. No work was done to attempt to explain these low results.

Reduction with zinc, decanting iodides through a filter, and dissolving and titrating the mercury gave results of 4.02, 4.08, 4.01, and 4.00 per cent.

The method was then used by six collaborators with the following results:

COLLABORATOR		HgI_2 *per cent*	
1	3.95	3.99	
2	4.01	3.95	
3	4.00	3.97	3.94
4	3.92	3.95	
5	4.01	3.91	
6	3.78	3.72	
Average	3.93		

In the case of No. 6, the ointment had melted in transit and the red mercuric iodide had separated. These results were on the remixed sample.

METHOD

Weigh 6–10 gram sample on cellophane or glassine paper and place in a separator. Add 50 ml. of ether to dissolve the ointment base. Extract with successive 10 ml. portions of 5% KI solution, swirling the contents of the funnel (3–4 shakeouts should be sufficient).

Filter each extract into a 250 ml. beaker. Add 0.25 gram of powdered zinc, and let stand, with frequent stirring, for 15 minutes. Decant through a Gooch and wash the residue thoroughly with successive portions of water, retaining most of the residue in the beaker.

Place the Gooch in the original beaker and dissolve the zinc with small portions of HNO_3 (1+5), using about 10 ml. in all. When the zinc is nearly all in solution add 10 ml. of HNO_3, and heat on the steam bath to effect complete solution of the mercury.

Add $KMnO_4$ solution (3–4%) to the solution on the steam bath until the purple color persists for 5 minutes. Discharge the color and dissolve the MnO_2 with just sufficient 3% H_2O_2. Cool, add 50 ml. of water, and titrate with 0.1 *N* NH_4SCN, using ferric alum indicator. 1 ml. of 0.1 *N* $NH_4SCN = 0.02272$ gram of HgI_2.

SUMMARY

The presence of iodides interferes in the determination of mercury. They are conveniently removed by reducing red mercuric iodide in potassium iodide solution with powdered zinc and decanting through a filter. The mercury can then be dissolved and titrated with thiocyanate. The presence of excess zinc does not interfere.

[5] *Ind. Eng. Chem., Anal. Ed.*, 10, 331 (1938).

A METHOD FOR THE DETERMINATION OF PROCAINE*

By JOHN R. MATCHETT and JOSEPH LEVINE (U. S. Bureau of Narcotics, Washington, D. C.)

A number of methods are available for the determination of procaine. They depend in general on (a) the formation of a colored compound subsequent to diazotization of the primary amino group;[1,2] (b) reaction of the substituted benzene ring with bromine[3] or iodine;[4] or (c) a direct determination of some other component of a mixture and estimation of procaine by difference.[5,6] Methods that are general for alkaloids, such as extraction with immiscible solvents and subsequent titration of the base, are also possibilities. The usefulness of the methods depends necessarily on a knowledge of the composition of the mixture under observation and of the interfering substances. Interfering substances are relatively numerous for each type of analysis and need not be further discussed.

The simple and precise method proposed here depends upon the distillation from alkaline solution of a basic substance equivalent in amount to the procaine present. Any substance that yields an alkaline distillate under these conditions will, of course, interfere. Such substances, however, are relatively few, and among those investigated include ammonium salts, stovaine, and beta-eucaine. Substances that do not interfere include lactose, chlorobutanol, heroine, morphine, cocaine, and codeine.

The method follows:

PROCEDURE

Weigh into a Kjeldahl flask a suitable sample of procaine or one of its salts. Dissolve in 150 ml. of distilled water and add 2 ml. of 50% NaOH. Quickly connect to a condenser and distil 100 ml. into a flask containing a measured excess of standard acid, extending the delivery tube below the surface of the solution. Remove the receiver, rinse the condenser with a little water, and titrate the excess acid with standard alkali solution, using methyl red as indicator. Each ml. of 0.1 *N* acid consumed is equivalent to 0.0236 gram of procaine or 0.0272 gram of procaine hydrochloride.

Representative results are given in the table.

COMMENT

In mixtures with certain alkaloids, notably codeine, the latter may be determined by extraction from the alkaline solution after distillation in the usual manner. In this work recovery of codeine was approximately 99 per cent.

The method provides an excellent test, in the negative sense, for the

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 30, 31, and November 1, 1939.

[1] Eissner, *Arch. Pharm.*, **268**, 322 (1930).
[2] Riegel and Williams, *J. Am. Chem. Soc.*, **48**, 2871 (1926).
[3] *Methods of Analysis, A.O.A.C.*, 1935, 574.
[4] Rae, *Pharm. J.*, **127**, 394 (1931).
[5] Milos, *Am. J. Pharm.*, **110**, 362 (1938).
[6] Schulek and Vastagh, *Arch. Pharm.*, **266**, 452 (1928).

presence of other alkaloidal salts admixed with procaine hydrochloride. If the presence of small quantities of cocaine are suspected, this test may occasionally be very useful. The procedure is simple and is readily

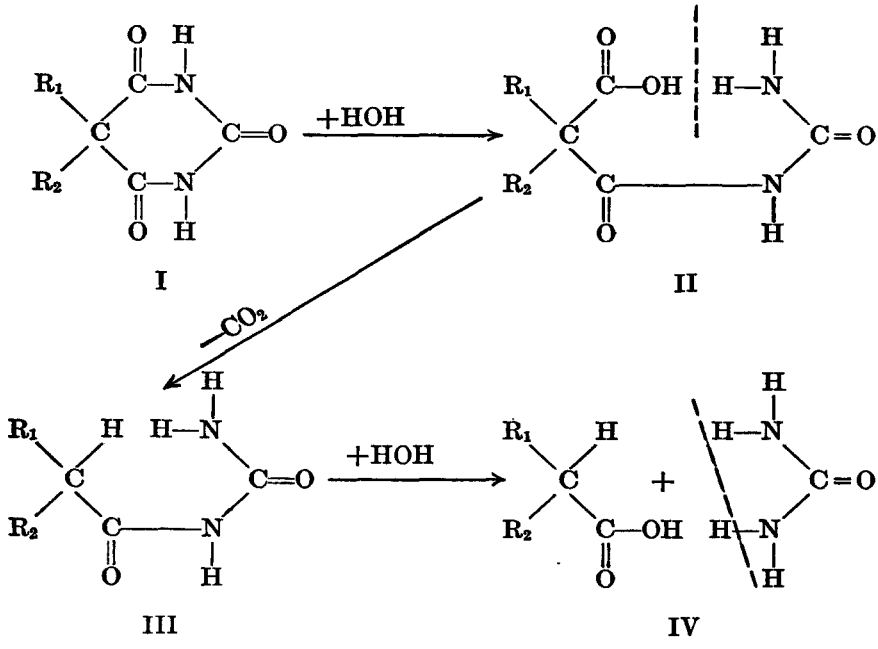
PROCAINE HCL	SUBSTANCE ADDED	WEIGHT	0.1 *N* HCl CONSUMED	RECOVERY
gram		*gram*	*ml.*	*per cent*
0.8174	—	—	29.90	99.72
0.9976	—	—	36.48	99.72
0.8024	Heroine Hydrochloride	0.648	29.36	99.75
0.9000	Codeine Sulfate	0.2226	33.07	100.17
0.7530	Codeine Sulfate	0.2815	27.66	100.13
0.9633	Codeine Sulfate	0.1930	35.07	99.24
1.0177	Codeine Sulfate Lactose	0.1917 1.00	37.51	99.94
0.9982	Cocaine Hydrochloride	0.488	36.68	100.17
0.5254	Chlorobutanol	0.8623	19.20	99.62
0.5175	Chlorobutanol	0.3860	18.95	99.82

adaptable to routine work. The use of micro apparatus and of more dilute solutions should make it even more suitable for rapid analysis. The procedure given is predicated upon samples equivalent to approximately 30 ml. of 0.1 *N* acid.

THE SEPARATION AND DETERMINATION OF PHENOBARBITAL IN SOLUTIONS

By Felice A. Rotondaro (U. S. Food and Drug Administration, Philadelphia, Pa.)

A number of workers have established the fact that the chief decomposition products of barbiturates in aqueous solutions are the correspondingly substituted malonuric acids, acet-ureides, and acetic acids. The essential features of the hydrolytic decomposition may be shown graphically by the following scheme:



(R_1 and R_2 may be any of the usual substituent groups, as ethyl-, phenyl-, etc.)

It has been found that diethyl-barbituric acid forms considerable amounts of the comparatively stable diethyl-malonuric acid (II) with a melting point of 161°–162° C.(6). When heated this compound gives off carbon dioxide and forms diethyl-acetyl-urea (III) with a melting point of 206°–207° C. Under similar conditions phenyl-ethyl-barbituric acid forms phenyl-ethyl-acetyl-urea (III), melting point 145°–148° C., which readily hydrolyzes further to phenyl-ethyl-acetic acid (IV) with a melting point of 44°–45° C. and a boiling point of 194°–195° C. (3, 6).

The usual procedures were followed in the separation of the various decomposition products; namely, filtering off the insoluble precipitates and extracting the filtrates with chloroform (2) or ether (5, 6). When filtrations and extractions of the solution were repeated at various *p*H levels, several fractions were separated (6). Therefore, it is apparent that all aqueous solutions of barbiturates may undergo appreciable decomposition, and their analysis requires that the barbituric acid found be free from its products of decomposition.

Warren (7) called attention to certain difficulties experienced in carrying out the analyses of elixir of barbital (9) and elixir of phenobarbital (10) by the N. F. VI method. While the revised N. F. VI (11) or A.O.A.C. (13) method eliminates the neutral decomposition product, acetyl-urea, by

discarding it along with "aromatics" or lubricants, no cognizance is taken of *acidic* products. That acidic products do often occur, however, is indicated by the fact that the melting point of the barbituric acid residue is usually low. This property has often been ascribed to the simultaneous extraction of some excipient, such as glycerin, when, in fact, the contaminant was phenyl-ethyl-acetic acid (melting point 44°–45° C.).

To better understand the nature of the decomposition products of phenobarbital the writer made the following experiment:

EXPERIMENTAL

Two grams of phenobarbital was boiled for 5 hours under a reflux condenser with 50 ml. of 0.2 *N* KOH solution. The cooled solution was made up to 100 ml. with water. A white flocculent precipitate was formed. A 50 ml. aliquot of the well mixed sample was placed in a separator and extracted with three 20 ml. portions of $CHCl_3$. The $CHCl_3$ extracts were filtered through $CHCl_3$-wet cotton into a tared evaporating dish. After the evaporation of the solvent the residue remained sirupy, even after it had been treated with several small portions of anhydrous ether. The residue thus obtained amounted to 8% of the sample taken. When it was dissolved in a little alcohol, diluted with water, and treated with one drop of 0.1 *N* HCl, it gave a definite pink color with methyl red indicator. A drop of 0.1 *N* NaOH changed the indicator back to yellow. After the mixture had stood a short time a white flocculent precipitate separated out. This was filtered off and dried, and its melting point was found to be 148°–150° C., which indicated phenyl-ethyl-acetyl-urea.

The alkaline solution remaining in the separator after the extraction of the acetyl-urea was made slightly acid to litmus with dilute HCl. Dry $NaHCO_3$ was next added in slight excess, and the undecomposed phenobarbital was extracted with five 20 ml. portions of $CHCl_3$. (That phenobarbital could be extracted from a bicarbonate solution had been shown by some previous work in an unsuccessful attempt to separate phenobarbital from phenolphthalein.) The phenobarbital residue obtained after the usual procedure of evaporating the solvent and treating with several additions of anhydrous ether was white and amounted to 45% of the sample. Its melting point (173°–176° C.) indicated it to be pure phenobarbital.

The residual bicarbonate solution in the separator was acidified once more with hydrochloric acid and extracted with three 20 ml. portions of $CHCl_3$. The clear colorless *sirupy* residue obtained amounted to 31.7% of the sample. Attempts to crystallize it failed. It was dissolved in a little alcohol, diluted with water, and titrated with 0.1 *N* NaOH, phenolphthalein being used as an indicator. The titration equivalent was 206. (Calculated titration equivalent of a 1:1 mixture of phenyl-ethyl-acetic and phenyl-ethyl-malonuric acid was 207.)

To check the probability of mistaking acidic decomposition products, due to their sirupy nature, for glycerin, which is a normal constituent of the N. F. VI elixir of phenobarbital, an "elixir" base was made according to the N. F. VI formula (10), including all ingredients except the phenobarbital. Invariably, only insignificant traces of a residue were obtained when the mixture was assayed by either of the N. F. VI methods. However, to eliminate the possibility of hydrolyzing some phenobarbital during the evaporation of the alcohol, as proposed by the revised N. F. VI method (11), the following assay method was used:

A 50 ml. sample was diluted with about 50 ml. of water in a separator, saturated with NaCl, and made acid with dilute HCl. The mixture was extracted to completion (6 or 7 times) with 25–35 ml. portions of $CHCl_3$ or about 35–50 ml. portions of ether if emulsions formed (the case with some sirups). The $CHCl_3$ extractions were filtered through $CHCl_3$-wet cotton into a beaker and the solvent was evaporated to a small volume (4–6 ml.) on a steam bath with the aid of a brisk current of air, care being taken that active ebullition of the solvent did not take place. The solution was transferred with the aid of a little $CHCl_3$ to a separator containing about 20 ml. of alkaline salt solution (0.5 *N* NaOH saturated with NaCl). The mixture was shaken thoroughly and the $CHCl_3$ was allowed to separate as completely as possible. The $CHCl_3$ was drawn off into a second separator containing about 3 ml. of 0.5 *N* NaOH and shaken well, and the $CHCl_3$ was allowed to separate completely, then drawn off through $CHCl_3$-wet cotton into a tared dish. The extraction was repeated with three or four more 20 ml. portions of $CHCl_3$. The combined $CHCl_3$ extracts were evaporated to dryness. (A few treatments with anhydrous ether greatly assists in completely expelling the last traces of the $CHCl_3$ solvent.) The residue was dried at 90°–100° C. for 10–15 minutes. Weight residue (A). (Residue (A) may contain non-volatile aromatic compounds plus any neutral decomposition products or drugs, as acetanilid, acetophenetidin, antipyrine, caffeine, etc.)

The alkaline wash water was combined with the alkaline salt solution. A piece of litmus paper was placed in the separator and acidified with dilute HCl. An excess of dry $NaHCO_3$ was added in small portions. The phenobarbital was completely extracted with 20–25 ml. portions of $CHCl_3$. Each portion was filtered successively through $CHCl_3$-wet cotton into a tared dish. The solvent was evaporated with the usual precautions. (The residue is usually sirupy due to traces of $CHCl_3$, which are tenaciously held until superheating causes the $CHCl_3$ to vaporize with explosive violence, thus causing losses by decrepitation. A few small additions of anhydrous ether materially shorten the time required to drive off the $CHCl_3$, minimize the loss by decrepitation, and leave dry, granular residues (B), which give melting points very close to theory.)

The $NaHCO_3$ solution in the separator was then cautiously acidified with dilute HCl. The mixture was extracted with three or four 20 ml. portions of $CHCl_3$ or other appropriate solvent and filtered, and the solvent was evaporated as usual. The residue (C) was examined for decomposition or acidic drugs, such as saccharin or aspirin, or salicylic, benzoic, or cinchoninic acids.

Table 1 shows typical results obtained with known quantities of phenobarbital (alcoholic solution) added to "elixir" base.

TABLE 1.—*Known quantities of phenobarbital added to "elixir" base*

SAMPLE	RESIDUE (A)	RESIDUE (B) (PHENOBARBITAL)			RESIDUE (C)
		THEORY	OBTAINED	RECOVERY	
		gram	*gram*	*per cent*	
1	Negligible	0.3000	0.2950	98.3	Trace
2	Negligible	0.3000	0.2965	98.8	Trace
3	Negligible	0.2500	0.2485	99.4	Trace
4	Negligible	0.2500	0.2475	99.0	Trace

When the revised method of assay (11) for elixir of phenobarbital was incorporated in the National Formulary (4th printing), an additional error

was introduced by the fact that saccharin was added as an ingredient of the "elixir." This ingredient is extracted from acid solution by chloroform along with the phenobarbital but no cognizance is taken of it in the assay although it accounts for 11.4 per cent of the residue. Warren (8) called attention to the possible significant contamination of barbital residues by gluside and suggested a method for its estimation by a sulfur determination and then an appropriate correction.

In the course of the work on the decomposition products one "elixir" encountered yielded a residue (C) from the acidified bicarbonate solution that amounted to about 10 per cent of the phenobarbital. This was tentatively assumed to be a decomposition product, because the phenobarbital was found to be short of the label declaration by about the same amount. However, the melting point of the residue was about 220° C. and therefore did not tally with known acidic decomposition products of phenobarbital. C, H, N, and S analyses, together with the melting point and crystalline characteristics of purified residue, indicated saccharin.

Another sample of an "elixir" was labeled to contain sodium benzoate, which was looked for in the final residue (C). The melting point of the residue obtained, however, was indefinite and indicated a mixture. A partial separation was made by extracting the residue with one portion of ether. The ethereal extract was then found to conform to benzoic acid while the comparatively ether-insoluble residue was found to conform to saccharin. It was later learned that saccharin is difficultly soluble in carbon tetrachloride (about 1:30,000) and that this solvent could be conveniently used for the separation of the usual acidic medicinal drugs from saccharin.

In order to establish the accuracy of the separation of acidic medicinals from phenobarbitals, the writer prepared alcoholic solutions of saccharin, aspirin, and cinchophen and salicylic and benzoic acids. Aliquots of these were then mixed with phenobarbital and assayed as described previously. Table 2 shows representative results obtained with phenobarbital and saccharin.

TABLE 2.—*Recoveries of phenobarbital and saccharin*

PHENOBARBITAL				SACCHARIN			
THEORY	OBTAINED	M.P.	RECOVERY	THEORY	OBTAINED	M.P.	RECOVERY
gram	*gram*	°C.	*per cent*	*gram*	*gram*	°C.	*per cent*
.1500	0.1505	175–178	100.3	0.0100	0.0095	223–225	95.0
.1500	.1485	173–175	99.0	.0100	.0102	220–222	102.0
.1500	.1495	175–178	99.7	.0100	.0098	222–224	98.0

Whenever the melting point of the residue obtained from the acidified bicarbonate solution does not indicate the substance suspected of being present, appropriate separations and determinations must necessarily be made.

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(8) *Ibid.*, 49.
(9) The National Formulary VI, p. 102.
(10) *Ibid.*, p. 123.
(11) *Ibid.*, Corrections in NF VI, 2nd List, p. 2.
(12) *Ibid.*, 4th printing, p. 123.
(13) *Methods of Analysis, A.O.A.C.*, 1935, p. 582.

DETERMINATION OF ACETANILID

By T. W. KETHLEY (Georgia Department of Agriculture, Atlanta, Ga.)

The National Formulary recognizes as an assay for acetanilid (1) the same type of method that is accepted by the Association of Official Agricultural Chemists (2). The determination involves acid hydrolysis of the acetanilid and the splitting out of acetic acid to form a water-soluble compound that can be separated from interfering materials and be brominated quantitatively with standard potassium bromide-bromate solution. This hydrolysis is time-consuming (4–6 hours) and it is necessary to keep close watch on the samples in order to prevent undue concentration of the sulfuric acid, which will cause sulfonation and oxidation.

Seidell (3), in 1907, first suggested the application of bromination to the determination of acetanilid. He used hydrochloric acid (1+4) for the hydrolyzing agent, and claimed excellent results. However, no data were given. In the same year, Turner and Vanderleed (4) presented a method for the alkaline hydrolysis of acetanilid for analytical purposes. Reclaire (5), in 1921, and Khaletskii and Mikryukova (6), in 1938, also published methods in which hydrochloric acid is the hydrolyzing agent.

It seemed impracticable to apply an alkaline hydrolysis to acetanilid in the presence of any other materials, but the use of hydrochloric acid as the hydrolyzing agent, with the subsequent bromination of the hydrolyzed acetanilid, seemed to offer possibilities.

All the methods cited previously were tried and were found to give fair results, but difficulties were encountered with those specifying boiling on the open flame as the method of heating, owing to the reduction of volume resulting from this treatment and the possibility of desiccation and destruction of the sample. Modifications of these methods that were tried involved refluxing, boiling gently with a larger volume, and heating

on the steam bath. Since the steam bath called for the least amount of work on the part of the analyst and of equipment outlay, this method was investigated further.

Although Seidell stated that one hour on the steam bath was sufficient to hydrolyze the acetanilid, he used a larger volume of solution than was thought feasible. The strength of acid used also seemed inadequate. The volume and concentration suggested by Reclaire (5) overcame these objections, and it was also found that one hour on the steam bath was sufficient time to hydrolyze as much as 0.4 gram of acetanilid. To determine the efficiency of the method, the writer checked it against the A.O.A.C. method, which constitutes an excellent criterion. In Table 1 are listed the results of this comparison. Column I gives the weight of acetanilid taken for the assay (U.S.P. acetanilid dried over sulfuric acid a minimum of 24 hours); Column II gives the values obtained using bromide-bromate solution standardized by the A.O.A.C. method (acetanilid as standard) to titrate samples hydrolyzed with hydrochloric acid; Column III gives the values obtained when the samples were also hydrolyzed with hydrochloric acid, but the bromide-bromate solution was standardized by the hydrochloric acid hydrolysis method (acetanilid as standard). These figures show the comparative values of the method as checked against the A.O.A.C. method, and against itself.

TABLE 1.—*Results on acetanilid by HCl hydrolysis*

WEIGHT	HYDROLYSIS BY HCl COMPARED WITH H_2SO_4	HYDROLYSIS BY HCl COMPARED WITH HCl
gram	*per cent*	*per cent*
0.2744	100.12	100.09
0.2298	100.40	100.46
0.2640	100.05	100.11
0.2506	99.89	99.96
0.2068	100.00	100.09
0.2409	99.96	100.02
0.2448	100.31	100.38
0.2669	100.35	100.42
0.2013	100.12	100.19
0.1353	99.96	100.03
0.2208	100.17	100.23
0.3093	99.80	99.88
0.3885	99.93	99.97
Average	100.09	100.14

SEPARATIONS

In determining acetanilid in mixtures, the separations outlined in *Methods of Analysis, A.O.A.C.* (2), are most satisfactory except in mixtures containing caffeine. The caffeine is separated from the hydrolyzed

acetanilid by extraction with chloroform; the aqueous solution of the hydrolyzed acetanilid is subsequently titrated with the bromide-bromate solution; and the caffeine is determined gravimetrically from the chloroform extract. However, if caffeine is not to be determined, it need not be separated, as it was found that caffeine had no effect on the titration of the aniline hydrochloride, provided the direct titration method was followed, i.e., titrating with bromide-bromate solution to the appearance of a slight yellow color, indicating the presence of excess bromine. The data that follow confirm the statement of Seidell (3) that caffeine does not interfere in this titration although he presented no data to support this contention.

Where the aniline hyrochloride is determined by the indirect method, which consists of addition of excess bromide-bromate, then potassium iodide solution, followed by back-titration of the excess iodine liberated by the bromine with thiosulfate, the caffeine has time and opportunity to take up, or otherwise combine with, a comparatively large quantity of the free bromine. It seems that this is true only when an excess of free bromine is present for an appreciable length of time. This is borne out by the following facts: If the indirect method is used in the presence of much caffeine, the result will be at least 10 per cent high (7); if caffeine is dissolved in hydrochloric acid of the strength used in the determination, bromide-bromate solution is added, and the mixture is allowed to stand a few minutes, the depth of color is appreciably diminished, indicating that some of the free bromine has been taken out of the solution. Further, if the direct titration is followed and the appearance of a slight yellow color is used as the end point, this end point will remain at least an hour if there is no caffeine; otherwise it will disappear within 4–5 minutes. The results show that four minutes is sufficient time to allow for the exact determination of acetanilid in the presence of caffeine without separation.

In Table 2 are listed the values obtained with hydrochloric hydrolysis

TABLE 2.—*Acetanilid results obtained by direct titration in the presence of 0.1 gram of caffeine*

WEIGHT	HCl HYDROLYSIS AND DIRECT TITRATION
gram	*per cent*
0.2533	99.94
0.2478	100.02
0.2252	100.10
0.2359	100.11
0.2425	99.79
0.2496	100.57
0.2810	100.13
0.2634	99.95
Average	100.07

and direct titration in the presence of 0.1 gram of caffeine. In each case 0.1 gram of caffeine was added to the sample, and titration was carried on without separation.

In 1910, Emery (8) stated that the hydrochloric acid method could not be used to determine both caffeine and acetanilid in mixtures because of the solubility of the corresponding hydrochlorides of both acetanilid and acetophenetidin. However, no such data were presented (9, 10, 11, 12).

Since the Handbook of Chemistry and Physics indicates that aniline hydrochloride is more soluble in water than is aniline sulfate, work was planned to investigate the solubility of the hydrochlorides in chloroform. Samples of acetanilid were weighed into 125 ml. flasks, 25 ml. of 4 *N* hydrochloric acid was added, and the samples were hydrolyzed on the water bath for 1 hour. They were then cooled, transferred to separators with a minimum of water, and extracted with three 50 ml. portions of chloroform, as specified in the A.O.A.C. method.

The aqueous portions were returned to the original flasks, placed on the water bath to remove any chloroform, cooled, and titrated with standard bromide-bromate solution. The recoveries are listed in Table 3.

TABLE 3.—*Recoveries of acetanilid after extraction with chloroform*

WEIGHT	RECOVERY
gram	*per cent*
0.2175	100.11
0.2347	100.05
0.2340	99.87
0.2369	100.08
0.2416	100.03
0.2458	99.48
0.2440	99.98
0.2836	99.89
0.2522	100.12
0.2311	99.89
Average	99.94

The solubility of the aniline hydrochloride is so slight (Table 3) that it is considered negligible. These results compare favorably with those given in Table 1, where the acetanilid was titrated without extraction with chloroform. The fact that the average is slightly lower than that in Table 1 (0.2%), might be accounted for by slight losses encountered in the double transfer and extraction, as well as the loss due to the solubility of the aniline hydrochloride in chloroform. To determine this solubility more carefully, the chloroform extracts from ten samples were collected, combined, and evaporated; and acid was added and titrated with standard bromide-bromate solution. This residue took up only 0.25 ml. of the solution, equivalent to a total of 2.8 mg., or 0.28 mg. per sample, which represents the

loss due to the solubility of the aniline hydrochloride in chloroform. This would represent an error in the acetanilid determination of 0.1 per cent, and certainly less than 1.0 per cent in a caffeine determination, depending on the size of the sample taken. This cannot be considered an appreciable error, particularly since the theoretical recovery of caffeine from acid aqueous solutions, when three portions of chloroform are used cannot be greater than 99.37 per cent. This theoretical value is obtained from the assumption that the extraction follows a straight-line value, and is based on the work of Emery (13) in 1921.

Although acetophenetidin had not been considered in the initial plan of this work, the results on the solubility of aniline hydrochloride in chloroform indicate the possibility of applying hydrochloric hydrolysis to this compound. To check the progress of hydrolysis, and to measure the solubility of the phenetidin hydrochloride in chloroform, the writer ran two series of twelve samples each, using 0.3 gram of acetophenetidin per sample. These samples were hydrolyzed with hydrochloric acid and extracted with chloroform, as directed in the A.O.A.C. method for separation of caffeine.

The chloroform from the twelve extractions was collected, distilled, and evaporated. The residue, which weighed 0.0400 gram in one case and 0.0440 gram in the other, or 0.0033 gram and 0.0036 gram per sample, respectively, consisted of a dark brown, resinous material, having a relatively low melting point. No crystalline iodide could be formed from this residue, which indicated that no unchanged acetophenetidin was present. However, the equivalent of about 0.0004 gram per sample as acetophenetidin was reformed on treatment with acetic anhydride and subsequent isolation. The bulk of the material seemed to be composed of addition or polymerization bodies, no positive identification of which could be made.

The slight evidence presented seems to indicate that although phenetidin hydrochloride is soluble in chloroform only to the extent of 0.4 mg. per sample, the hydrochloric acid procedure tends to decompose (or more likely, cause additions to) this compound and make the method unsuitable for the separation of mixtures of acetophenetidin and caffeine.

The details of the method formulated by the writer follow:

METHOD

Weigh 0.2–0.3 gram of acetanilid and extract with $CHCl_3$ to dissolve the acetanilid. If other $CHCl_3$-soluble substances are present or are to be determined, follow the separation procedures outlined in *Methods of Analysis, A.O.A.C.*, 1935. Place the $CHCl_3$ extract in a 125 ml. Erlenmeyer flask, evaporate on the steam bath almost to dryness, add 25 ml. of 4 *N* HCl, and heat the mixture on the steam bath for 1 hour. Then cool the sample and wash down the insides of the flask with 10 ml. of distilled water.

If caffeine is to be determined, extract it from the hydrolyzed sample as directed in the A.O.A.C. method. Titrate with the bromide-bromate solution. Shake the flask slowly, in a rotatory motion until the end point is approached, as this will give

a flocculent precipitate that will separate from the fluid phase easily and make reading of the end point more accurate. As the end point is approached, add the solution dropwise, with violent agitation, in order to insure bromination. In the presence of caffeine, use particular care not to pass the end point, as the caffeine will tend to take up any large excess of free bromine and lead to confusion.

Prepare the bromide-bromate solution or any other reagent used in this analysis, with the exception of the HCl as directed in the A.O.A.C. method. The HCl solution, which is approximately 4 *N*, is most easily prepared by making 400 ml. of HCl to one liter with water.

SUMMARY

(1) A method is proposed for the determination of acetanilid to be substituted for the A.O.A.C. and N. F. sulfuric acid hydrolysis.

(2) The method requires only one hour on the steam bath, practically no attention on the part of the analyst, and no special equipment.

(3) The accuracy of the method was checked against the A.O.A.C. and N.F. methods and found to be in excellent agreement.

(4) Acetanilid can be titrated directly in the presence of caffeine.

(5) The solubility of aniline hydrochloride in chloroform is not sufficient to create an appreciable error in the determination of caffeine in the presence of acetanilid by this method.

(6) This method is not applicable in the presence of acetophenetidin.

ACKNOWLEDGMENTS

Appreciation is expressed to the men at the Atlanta Station, F.D.A., for their advice and help, and to the reviewers who examined the original paper on which this work is based for their pertinent suggestions.

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(2) *Methods of Analysis, A.O.A.C.*, 1935, 541–547.
(3) A. SEIDELL, *J. Am. Chem. Soc.*, **29**, 1091 (1907).
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(9) ———, *Ibid.*, **122**, 100 (1909).
(10) ———, *Am. J. Pharm.* **81**, 3 (1909).
(11) ———, *Bur. Chem. Bull.* **137**, 183 (1911).
(12) ———, *Ibid.*, **152**, 236 (1912).
(13) ———, and C. C. WRIGHT, *J. Am. Chem. Soc.*, **43**, 2323 (1921).

ASSAY OF LEAD OLEATE PLASTER AND OINTMENT OF LEAD OLEATE

By HENRY M. BURLAGE* (University of North Carolina School of Pharmacy, Chapel Hill, N. C.)

Until recent years lead oleate plaster and the ointment of lead oleate

* The writer wishes to acknowledge with thanks the assistance of Herman O. Thompson, Lafayette, Ind., in carrying out some of the preliminary experiments on the assay of the plaster.

were quite widely used, but they are now of declining importance. However, both preparations are still official in the National Formulary, and no method of assay has been recognized in the United States Pharmacopoeia, in the National Formulary, or in *Methods of Analysis, A.O.A.C.*, 1935. Since samples of the plaster and the ointment were found to vary considerably in physical properties, and especially in appearance and consistence, the writer decided to study proposed methods of assay with a view to improvement.

The chief action of the plaster and the ointment depends upon the properties of the lead present as the oleate, and the plaster has been evaluated on the basis of the equivalent of lead monoxide in the sample.

Three methods are given in the literature for the determination of lead as lead oxide in lead oleate plaster: (a) the Harrison and Watt method[1] which is an ignition and gravimetric procedure; (b) the Wetherell method,[2] a precipitation and gravimetric procedure with oxalic acid; and (c) the Babitsch method,[3] a volumetric assay.

The methods of Wetherell and Babitsch were studied. Because of the variable moisture content of the plaster, Wetherell found it necessary to dry the sample before assaying and to report results on the dry basis. He calculated the quantity of moisture as the loss after three hours of heating in an oven at 100°–105° C. At the conclusion of this period the loss per hour was negligible. This observation was substantiated in the present study. The two methods failed to give concordant results, and in general Wetherell's method gave lower values. The following procedure, which embodies features of both methods, especially that of Babitsch, was found to give reliable results with the plaster as well as the ointment.

METHOD

Accurately weigh approximately 1 gram of the plaster (or 2 grams of the ointment) into a tared 125 ml. Erlenmeyer flask. Determine the moisture content by drying in an oven at 100°–110° C. for 3 hours. Add 10 ml. of 36% acetic acid, heat the mass on a water bath until it liquefies, add 25 ml. of hot water, and shake vigorously. Allow to cool, and if necessary chill and puncture the solidified layer of fatty material. Transfer the aqueous solution of the lead salt to a 150 ml. Erlenmeyer flask, filtering the liquid through a small pledget of cotton. Repeat the extraction with 5 ml. of the acid and 25 ml. of hot water 3 more times. Heat the combined aqueous extracts to boiling, add the equivalent of 50 ml. of 0.1 *N* oxalic acid, shake well, and allow to cool. Filter, and wash the flask and precipitate on the filter carefully with a total of 125 ml. of water in 25 ml. portions. Allow the precipitate to drain well and transfer the paper and precipitate to the original 250 ml. Erlenmeyer flask. Add 10–15 ml. of 10% H_2SO_4 and about 75 ml. of distilled water and heat almost to boiling; then add 10 ml. more of the diluted acid and titrate the mixture at about 70° C. with 0.1 *N* $KMnO_4$. 1 ml. of 0.1 *N* $KMnO_4 = 0.01116$ gram of PbO.

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[1] *Yearbook Pharm.*, 1908, 527–30.
[2] *Quart. J. Pharm. Pharmacol.*, 8, 461–68 (1935).
[3] *Arch. Pharm.*, 271, 446–48 (1933).

PREPARATION OF THE SAMPLES

Sample A.—Lead oleate plaster was carefully prepared in the laboratory according to the directions in National Formulary VI, p. 131.

Sample B.—Lead oleate ointment was prepared from Sample A according to the directions in National Formulary VI, p. 425.

Sample C.—This sample was a commercial product labeled "Diachylon Ointment, N. F."

The results of analysis are shown in the table.

SAMPLE	MOISTURE		PbO ON THE DRY BASIS
	per cent		*per cent*
A-1	3.55		32.42
A-2	4.52		32.31
A-3	5.07		32.49
		Av.	32.41
B-1	4.36		14.54
B-2	4.07		14.67
B-3	4.48		14.70
B-4	3.77		14.63
		Av.	14.64
C-1	1.34		13.61
C-2	1.58		13.61
C-3	1.82		13.63
		Av.	13.62

Many preliminary experiments were also made with Wetherell's and Babitsch's methods in an attempt to arrive at concordant results. The method of removing fatty material by means of solvents, especially ether and chloroform, did not give satisfactory results. Residual titrations of oxalic acid with potassium permanganate, after the removal of the fat and the lead as the oxalate, yielded erroneous results because of incomplete removal of unsaturated fatty constituents before and after precipitation.

SUMMARY

An improved procedure is proposed for the determination of lead in lead oleate plaster and lead oleate ointment (National Formulary) as lead monoxide. Features of the Wetherell and the Babitsch methods were utilized.

Samples of the plaster and of the ointment were found to vary considerably in moisture content.

THE ASSAY OF EPHEDRINE PREPARATIONS
(Especially of Those Containing Aldehydes)

By E. H. GRANT (U. S. Food and Drug Administration, Boston, Mass.)

In connection with his work as Associate Referee on Ephedrine in Jellies, the writer made several observations not strictly concerned with jellies. This work is reported as a separate paper.

It is difficult at times to recover ephedrine alkaloid by the A.O.A.C. method from oily solutions or those made from a petroleum jelly base, and chemists have sought to find a more direct method of assay.

Ephedrine alkaloid is quite soluble in water, and on shaking an oil solution of ephedrine, such as Compound Ephedrine Inhalant, N. F. VI, with water, some alkaloid dissolves in the water. Attempts were made to determine the alkaloid by direct titration, as follows:

> Weigh 5 grams of inhalant into a small, glass-stoppered flask, add 5 ml. of water and a drop or two of bromothymol blue indicator solution, and titrate with 0.02 *N* H_2SO_4. Moderately violent shaking is necessary towards the end of the titration.

In the absence of other basic substances, such as ammonia, this will measure the free ephedrine. The titrated mixture may be washed with ether and water into a separator, the aqueous portion drawn off, the ethereal layer washed with water, and the aqueous fractions washed with ether and the solution evaporated. Neutral ephedrine sulfate results, the purity of which may be further investigated.

Ephedrine combines with certain aldehydes to form loose compounds. For example, it is said to form benzalephedrine with benzaldehyde. Some of these aldehydes, for example, benzaldehyde, may result from the decomposition of ephedrine, and under such circumstances they would combine with a portion of the undecomposed ephedrine. The aldehyde compounds of ephedrine are insoluble in water and would not be titrated in the last-mentioned method. They are, however, weakly basic in nature and follow ephedrine in the A.O.A.C. method.

The writer and others of his acquaintance have occasionally encountered certain similar peculiar compounds in the assay of inhalants. Generally they are so insoluble in water that a colored end point cannot be obtained in the titration, but one must rely on the first appearance of a permanent cloud. On long standing, the compound will dissolve enough to affect the indicator.

The free ephedrine and ephedrine-aldehyde compound may be separated from each other by titrating the free ephedrine direct, washing out the ephedrine sulfate as previously suggested, then adding an excess of acid, shaking and proceeding as in the A.O.A.C. method, evaporating the final ethereal solution to dryness without the application of heat.

An old sample of ephedrine inhalant showed 0.792 per cent of ephedrine by the A.O.A.C. method. A titration of the free ephedrine corresponded to 0.68 per cent. A titration of the separated aldehyde compound corresponded to 0.312 per cent of ephedrine alkaloid. On evaporating the solution from the first titration to dryness, the weight of ephedrine sulfate corresponded to 0.674 per cent of ephedrine alkaloid. On re-extracting the aldehyde compound, a residue corresponding to 0.296 per cent of the sample was obtained. It was a white, apparently crystalline, compound with an aromatic odor.

It has been suggested that the ephedrine in inhalants be extracted with dilute hydrochloric acid, that this solution be evaporated to drive off the excess acid and that the ephedrine hydrochloride be weighed. This is evidently not applicable to any inhalant containing any of these aldehyde compounds, because the aldehyde portion of the molecule follows the ephedrine and, unless decomposed during evaporation, would be weighed along with the ephedrine hydrochloride. For example, in the sample cited above, the A.O.A.C. method showed 0.792 per cent of ephedrine, whereas this suggested method would have shown in the neighborhood of 0.674 per cent plus 0.296 per cent, or 0.97 per cent of ephedrine.

On adding an excess of benzaldehyde or cinnamic aldehyde to ephedrine inhalant, no free ephedrine could be titrated by the described method. Only a minute amount of the aldehyde complex is soluble in mineral oil and the inhalant clouds if too much aldehyde is added. If none of this insoluble matter has settled out, no difficulty is encountered in determining ephedrine by the official method and the results are the same as if no aldehyde were added. (See *This Journal*, **14**, 328 (1931), where cinnamon oil was used.)

The benzaldehyde-ephedrine and cinnamic aldehyde-ephedrine compounds so prepared are not so insoluble in water as to interfere with the end point; therefore, neither of them is identical with the insoluble compound often encountered in commercial samples of deteriorated ephedrine inhalant.

After titration, the solutions of the neutralized aldehyde-ephedrine complexes prepared, respectively, from benzaldehyde and cinnamic aldehyde, were each made ammoniacal and extracted repeatedly with washed ether. The ethereal solutions were then washed with water to remove ammonia. About an equal volume of water was then added, also a drop of indicator. This showed the water layer to be neutral in the beginning. The complexes gradually decomposed to yield free ephedrine, which dissolved in the water layer; 0.02 *N* sulfuric acid was added to exact neutrality from time to time, with shaking. At the end of a week, each of the complexes had decomposed completely. The aqueous solutions were then evaporated to dryness, yielding pure ephedrine sulfate in each case, as shown by polarization and assay.

No success was experienced in preparing the above-mentioned compound found in deteriorated inhalants by exposing some of a fresh batch of inhalant, made by the same formula as used in the old batch, to sunlight.

Ephedrine in inhalants may also be determined by shaking with a measured amount of standard acid, in divided portions, followed by water, and titrating the excess acid with standard sodium hydroxide solution. This method is simpler than the official method and eliminates several steps where ephedrine may be lost. Ammonia, amines, and water-soluble acids would interfere; oil-soluble acids and aldehydes would not. Because of such interfering substances, this method is not recommended for official study, but some chemists may find it valuable for a rapid or supplemental method.

QUANTITATIVE CHARACTERISTICS OF THE NICOTINE COLOR REACTION WITH CYANOGEN BROMIDE AND β-NAPHTHYLAMINE

By L. N. MARKWOOD (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

The writer recently described an adaptation of Barta and Marschek's colorimetric method for nicotine[1] in the determination of nicotine deposits on apples.[2] In this method the color is developed from nicotine through the König reaction[3] by means of cyanogen bromide and β-naphthylamine.

The characteristics of the reaction, such as the color developed and the time required for maximum color development, are affected by the hydrogen-ion concentration and by salts. In certain applications of the method an extraneous salt is formed when the test solution is prepared. For example, in the method for apples a nicotine insecticide on the apple skin is dissolved with sodium hydroxide. The alkali must then be neutralized before the color reactants are applied, and it is therefore necessary to make a choice of acid for neutralization. Similarly, in fumigation tests nicotine vapor is absorbed in an acid solution, which must be neutralized before the test is continued. In this case the choice of acid for the absorption must be considered.

The first consideration is the optimum hydrogen-ion concentration, that is, the *p*H value that will yield the greatest color development. Other factors to be determined are the effect of the different acids used in neutralization on the development of color at the optimum *p*H value and of varying concentrations of salts and the range of nicotine concentrations applicable to Beer's law.

In the previous paper[2] on the sodium hydroxide-acetic acid system the

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[1] *Mezögazdasági Kutatások*, **10**, 29 (1937).
[2] *This Journal*, **22**, 427 (1939).
[3] König and Bayer, *J. prakt. Chem.*, **83**, 325 (1911).

writer reported that the phenolphthalein neutralization point is the point of maximum color development, a conclusion reached from studies made with the visual, neutral-wedge photometer.[4] In the experiments described here all the measurements were made with a photoelectric photometer of the double-cell type substantially as described by Brice,[5] with the modification that test tubes were used as the cells, in the manner adopted by Evelyn.[6] These tubes are $7 \times \frac{7}{8}$ inches. A dark blue-green filter (Corning 430), consisting of two pieces of glass, each 3.55 mm. thick, was used.

The optimum point for the sodium hydroxide-acetic acid system was found to be slightly on the alkaline side of the phenolphthalein end point, and similar results were obtained with hydrochloric and sulfuric acids. For reasons mentioned later, however, neutralization to the phenolphthalein end point is still retained.

EXPERIMENTAL

Determination of optimum pH.—Test solutions were prepared by pipetting 5 ml. aliquots of a standard solution containing 100 micrograms of nicotine per ml. into 100 ml. volumetric flasks, adding exactly 10 ml. of approximately 0.1 *N* sodium hydroxide and variable quantities of approximately 0.1 *N* acetic, hydrochloric, or sulfuric acid, and making up to volume. The solutions then contained 5 micrograms of nicotine per ml. plus the quantity of salt formed by neutralization and any excess base or acid.

The color was developed by adding 1 ml. of aqueous cyanogen bromide solution and 5 ml. of alcoholic β-naphthylamine solution to 5 ml. of the test solution, as described in the earlier paper.[2] The solutions were allowed to stand in the dark for the period required to give maximum color, and measurements of light transmission were then made. Transmission and color are inversely related; i.e., the greater the color the less the transmission.

Measurements of *p*H were made with a Beckman meter (glass electrode).

The data in Table 1 relate to experiments made with the three acids. The minimum light transmission for acetic acid occurred at a *p*H of 9.8, for hydrochloric acid at 10.1, and for sulfuric acid at 10.0. No significance should be attached to the slight differences between these values, since the solutions were not bracketed closer than 0.2 ml. of acid. Accordingly, it is concluded that a *p*H of about 10 gives the maximum color.

Theoretically, then, a more alkaline indicator than phenolphthalein should be used. Satisfactory alkaline indicators are rare. Thymolphthalein, possibly the best, has a transition point about 1 *p*H unit higher than phenolphthalein. The end point is draggy, however, and the gain in sensitivity is slight. The use of phenolphthalein was therefore retained.

[4] Clifford and Wichmann, *This Journal*, **19**, 130 (1936).
[5] *Rev. Sci. Instruments*, **8**, 279 (1937).
[6] *J. Biol. Chem.*, **115**, 63 (1936).

TABLE 1.—*Relation of pH value and light transmission in the nicotine color reaction when various acids are used for neutralization*
(Nicotine concentration, 5 micrograms per ml.; 10 ml. of 0.1 *N* NaOH and indicated quantity of acid per 100 ml.)

ACETIC ACID				HYDROCHLORIC ACID				SULFURIC ACID			
VOLUME	pH	LIGHT TRANSMISSION	COLOR DEVELOPED (1 HOUR)	VOLUME	pH	LIGHT TRANSMISSION	COLOR DEVELOPED (3 HOURS)	VOLUME	pH	LIGHT TRANSMISSION	COLOR DEVELOPED (2 HOURS)
ml.		*per cent*		*ml.*		*per cent*		*ml.*		*per cent*	
0	11.4	99.8	Colorless	0	11.5	99.7	Colorless	0	11.5	99.8	Colorless
2	11.35	99.8	Colorless	2	11.45	99.7	Colorless	2	11.45	99.8	Colorless
4	11.3	99.7	Colorless	4	11.35	99.7	Colorless	4	11.3	99.7	Colorless
6	11.2	90.2	Yellow	6	11.3	93.0	Sl. yellow	6	11.3	94.9	Very sl. yellow
8	11.1	78.5	Yellow	8	11.1	84.7	Sl. orange	8	11.15	87.6	Sl. yellow
9.1	10.9	72.3	Orange	10	10.85	75.2	Pink	10	10.9	80.3	Orange
9.4	10.8	71.3	Pink	10.5	10.75	73.7	Pink	10.5	10.8	78.0	Orange
9.7	10.7	69.5	Pink	10.8	10.65	72.8	Pink	10.8	10.75	77.2	Pink
10.0	10.5	69.1	Pink	11.1	10.5	72.0	Pink	11.1	10.6	76.3	Pink
10.3	10.3	68.2	Pink	11.4	10.4	71.0	Pink	11.4	10.4	75.6	Pink
10.6	9.8	67.4*	Pink	11.7	10.1	70.2*	Pink	11.7	10.3	74.9	Pink
10.9†	7.9	68.6	Pink	12.03	9.7	70.5	Pink	12.0	10.0	74.7*	Pink
11.2	6.3	70.7	Yellow	12.33	8.95	71.9	Orange	12.25	9.45	75.0	Pink
				12.53†	7.75	74.2	Orange	12.50†	7.85	78.3	Orange
				12.73	5.4	77.2	Yellow	12.75	5.65	81.3	Yellow
				12.93	3.75	77.7	Yellow	13.0	3.85	82.3	Yellow
				13.13	3.35	78.2	Yellow	13.25	3.5	82.7	Yellow

* Minimum transmission.
† Phenolphthalein neutralisation point

The solutions at *p*H 10 are pink in the presence of phenolphthalein, an inadmissible condition for measuring yellow, orange, or red. They must be decolorized by neutralization. In the case of acetic acid there is very litttle difference between the transmission at the neutralization point (68.6) and the minimum point (67.4). With hydrochloric and sulfuric acids the differences are greater (for HCl, 74.2–70.2; for H_2SO_4, 78.3–74.7). These differences indicate some loss of sensitivity if the work is done at the phenolphthalein neutralization point, and yet they are not large enough to warrant rejection of the phenolphthalein basis of standardization.

Since the foregoing basis was established, a paper by Borozdina[7] has been noted. He divided an acid solution containing nicotine into two equal parts, titrated one-half with sodium hydroxide to the methyl red end

TABLE 2.—*Effect of light transmission of various acids used for neutralizing alkali in nicotine color reaction*

ACID	CONCENTRATION OF SALT FORMED (CALC'D)	LIGHT TRANSMISSION (PER CENT)								
		NICOTINE CONCENTRATION 5 MICROGRAMS PER ML.					NICOTINE CONCENTRATION 25 MICROGRAMS PER ML.			
		½ HR.	1 HR.	2 HRS.	3 HRS.	4 HRS.	1 HR.	2 HRS.	3 HRS.	4 HRS.
	gram/100 ml.									
None	—	—	75.3	72.0	71.1	71.6	31.8	26.7	25.3	25.6
Hydrochloric	0.073	—	74.3	72.1	71.0	71.6	31.4	26.3	25.6	25.8
Sulfuric	0.089	—	75.1	73.8	74.3	76.8	32.0	28.9	29.4	32.2
Acetic	0.103	63.8	63.4	63.7	64.2	65.8	14.8	13.2	13.4	14.0

point, and added the volume of alkali so found to the other half, which he then treated with cyanogen bromide and aniline. This procedure is obviously a means of eliminating color due to indicator. As seen from Table 1, the methyl red end point (about *p*H 5.2) would give a value farther from the minimum than that obtained at the phenolphthalein end point.

Changes in the *p*H values are reflected in the colors developed. Above 11.3 the reaction is completely inhibited; that is, no color develops. Between approximately 11.3 and 11.1 yellow is produced, and between approximately 11.1 and 10.9 the color is orange. Thereafter it is pink to the phenolphthalein neutralization point, although in the solutions neutralized by the mineral acids it is more orange than pink. In the acid region the color is again yellow.

Selection of acid giving greatest sensitivity.—The acids in Table 1 cannot be accurately compared, because the runs were made at different times.

[7] *Vsesoyuz. Inst. Tabach. i Makhoroch. Prom.*, No. 133, 158 (1937).

TABLE 3.—*Relation of concentration of $NaC_2H_3O_2$ and light transmission in nicotine color reaction*

CONCENTRATION OF $NaC_2H_3O_2$ (GRAMS/100 ML.)	LIGHT TRANSMISSION (PER CENT)													
	NICOTINE CONCENTRATION 5 MICROGRAMS PER ML.								NICOTINE CONCENTRATION 25 MICROGRAMS PER ML.					
SERIES A	½ HR.	¾ HR.	1 HR.	2 HRS.	3 HRS.	4 HRS.			½ HR.	¾ HR.	1 HR.	2 HRS.	3 HRS.	4 HRS.
0.0	81.2	79.6	77.9	75.7	75.1*	75.4			41.6	38.5	36.3	32.2	30.7*	30.8
.25	66.5†	66.3†*	66.6†	69.0†	70.4†	71.7†			22.2†	17.3†*	17.6†	19.2†	20.6†	21.5†
.5	68.0	67.6*	68.5	72.2	73.4	75.0			22.9	18.6*	19.4	21.9	23.9	24.9
1.0	71.5	70.2*	71.3	75.4	76.5	78.0			25.6	21.6*	22.2	26.4	29.0	30.9
2.0	74.4	74.0*	74.5	78.2	79.8	81.0			29.2	25.4*	26.3	31.3	35.2	37.3
4.0	77.7	77.1*	77.7	81.1	82.7	84.3			34.7	30.6*	31.7	37.8	42.1	44.2
6.0	78.8	78.5*	79.2	82.9	84.4	86.2			38.6	34.2*	35.3	41.7	46.2	49.0
8.0	80.9	80.0*	80.8	84.6	86.2	87.5			42.6	37.6*	38.5	45.0	50.0	53.2
SERIES B	¼ HR.	½ HR.	1 HR.	1½ HRS.	1½ HRS.	1¾ HRS.	2 HRS.	2½ HRS.	½ HR.	¾ HR.	1 HR.	1½ HRS.	2 HRS.	2½ HRS.	3 HRS.
0.0	84.6	81.0	79.3	77.8	77.3	76.9	76.4	75.8	46.9	—	34.7	—	32.3	—	30.3*
.025	74.8	72.2	71.3	70.2	69.9	69.6	69.3	68.8*	29.9	25.5	22.6	20.7	19.9	19.5*	20.2
.05	69.8	67.7	67.2	66.1	65.9	65.6*	65.9	66.2	25.5	21.4	18.9	17.8	17.6	17.4*	18.0
.1	66.6	64.7	64.2	64.0*	64.1†	64.2†	64.3†	64.7†	22.9	19.0	18.0	17.5†	17.2†	16.9†*	17.8†
.2	64.7†	63.6†	63.2†*	63.8†	64.2	64.5	64.7	65.1	21.8†	18.3†	17.9†*	18.1	18.3	18.8	19.8
.5	65.0	64.3*	64.6	65.2	65.9	66.5	67.2	68.2	22.3	19.4*	19.8	21.6	22.5	23.6	25.0
1.0	67.3	66.7*	67.2	68.4	69.1	69.9	70.7	71.6	23.9	21.3*	22.7	25.1	26.6	28.2	30.1

* Minimum transmission at a given concentration of $NaC_2H_3O_2$.
† Minimum transmission at a given time.

Accordingly, another experiment was conducted in which the neutralized solutions of the different acids, prepared as before, were tested in a single run. Two concentrations of nicotine were tested, 5 and 25 micrograms per ml., to obtain information valid over a wide range. The results are shown in Table 2. The lowest transmission resulted when acetic acid was used, after 1 hour at the 5 microgram level and 2 hours at the higher level. When hydrochloric acid was used, the minimum value occurred in 3 hours. There was very close agreement between the values for this acid and those

TABLE 4.—*Relation of concentration of NaCl and light transmission in nicotine color reaction*

NaCl CONCENTRATION (GRAMS/100 ML.)	LIGHT TRANSMISSION (PER CENT)							
	NICOTINE CONCENTRATION 5 MICROGRAMS PER ML.				NICOTINE CONCENTRATION 25 MICROGRAMS PER ML.			
SERIES A	1 HR.	2 HRS.	3 HRS.	4 HRS.	1 HR.	2 HRS.	3 HRS.	4 HRS.
0.0	80.4	78.4	78.1*	78.7	39.6	35.5	35.3*	36.0
.25	80.7	78.6	78.5*	79.0	41.2	36.9	36.7*	38.5
.5	81.2	78.7*	78.9	79.5	42.5	37.5*	37.9	39.3
1.0	81.4	78.8*	79.5	79.9	43.7	38.7*	38.8	40.2
2.0	81.4	78.7*	79.8	80.5	44.4	39.5*	39.7	41.7
4.0	81.3	78.7*	79.8	80.8	45.4	40.6*	40.8	42.7
6.0	81.4	79.0*	79.9	80.9	46.3	41.2*	41.5	43.4
8.0	81.4	78.9*	79.9	80.8	47.0	42.0*	42.3	43.9
SERIES B								
0.0	78.9	76.6	76.3*	77.1	36.7	32.6	32.2*	33.8
.025	79.2	76.9	76.7*	77.3	37.1	33.1	32.7*	34.1
.05	79.3	77.0	76.8*	77.4	37.5	33.7	33.3*	34.6
.1	79.5	77.3	77.0*	77.7	37.8	34.0	33.7*	34.9
.2	79.6	77.4	77.3*	78.1	38.0	34.3	34.0*	35.5
.5	79.6	77.5*	77.7	78.7	38.3	34.5*	34.9	36.5
1.0	79.7	77.6*	77.8	79.2	38.7	35.6*	35.7	37.8

* Minimum transmission at a given concentration of NaCl.

for an aqueous solution of nicotine without alkali or acid. The least sensitivity was given by sulfuric acid, and the minimum value occurred in 2 hours. It is shown, therefore, that neutralization with acetic acid gives the greatest sensitivity.

Effect of salts at various concentrations.—The effect on the light transmission of varying concentrations of the salts produced by neutralization with each of the three acids was next studied. The two concentrations of nicotine were used as before. The concentration of each salt was varied over a wide range, 0–8 grams per 100 ml. (series A), which no doubt is wider than will occur in practice. A second series, in concentrations from 0 to 1 gram per 100 ml. (series B), was studied separately to give greater detail in the region most likely to be used. Although the use of these salts

is not exactly equivalent to the working operation of neutralizing alkali with acid, any differences therefrom are only minor.

The results for sodium acetate are shown in Table 3. The optimum developing time in the range 0.25–8.0 grams is 45 minutes. As the concentration of salt decreases from 0.25 to 0.025 gram the optimum time increases to 2½ hours, although at the higher nicotine level this period is indicated up to 0.1 gram of salt. Replications of the experiment have confirmed this

TABLE 5.—*Relation of concentration of Na_2SO_4 and light transmission in nicotine color reaction*

Na_2SO_4 CONCENTRATION (GRAMS/100 ML.)	LIGHT TRANSMISSION (PER CENT)							
	NICOTINE CONCENTRATION 5 MICROGRAMS PER ML.				NICOTINE CONCENTRATION 25 MICROGRAMS PER ML.			
	1 HR.	2 HRS.	3 HRS.	4 HRS.	1 HR.	2 HRS.	3 HRS.	4 HRS.
Series A								
0.0	81.4	77.8	77.1*	78.1	38.2	31.5	29.8*	30.7
.25	81.6	79.3*	80.2	82.7	38.8	33.9*	34.3	37.6
.5	81.7	80.2*	81.3	84.0	39.2	34.5*	35.8	40.1
1.0	81.7	80.7*	82.1	85.0	39.4	35.4*	37.3	41.6
2.0	81.7	80.9*	82.5	85.4	39.7	36.0*	38.4	43.3
4.0	81.7	81.1*	82.7	85.6	40.3	37.1*	39.7	44.7
6.0	81.7	81.3*	83.0	85.9	40.7	37.5	40.1	45.1
8.0[a]	—	—	—	—	—	—	—	—
Series B								
0.0	81.2	78.6	77.4*	77.8	40.8	33.2	32.0*	32.5
.025	81.4	79.1	78.2*	78.5	41.2	34.3	33.5*	33.9
.05	81.6	79.5	78.7*	79.2	41.8	35.4	34.8*	36.1
.1	82.0	80.0	79.2*	79.9	42.6	36.6	36.2*	37.6
.2	82.1	80.2	79.8*	81.0	43.0	37.6	37.5*	39.6
.5	82.2	80.7*	81.2	82.4	43.3	38.6*	39.2	42.3
1.0	82.3	80.9*	81.5	83.2	43.8	39.5*	40.5	45.4

* Minimum transmission at a given concentration of Na_2SO_4.
[a] Solutions turbid owing to precipitation of Na_2SO_4 by alcohol.

behavior. At the concentrations up to 0.1 gram the optimum time is only slightly less than it is in the absence of salt, for the time without salt is shown as 3 hours, and closer bracketing would reveal it to be 3½ hours. The conditions for producing maximum sensitivity with sodium acetate are a salt concentration of 0.2 gram and a developing period of 1 hour. The data show clearly that this salt in proper concentration markedly sensitizes the reaction. With 0.5 gram or less of the salt the transmissions are always less than in its absence.

The results for sodium chloride are shown in Table 4. A salient point here is that the concentration of salt has relatively little effect on the transmission. At the lower nicotine level there is a slight upward trend

to a salt concentration of about 1.0 gram, beyond which the transmission remains fairly stable. At the higher nicotine level the transmission rises continuously by small increments as the salt concentration increases.

In contrast to the behavior with sodium acetate, the reaction is less sensitive in the presence of any quantity of sodium chloride than in its absence. The transmissions at the lower nicotine level, however, are but slightly higher than for plain aqueous solutions. For all but very exact work, any concentration of salt up to 1.0 gram may be allowed at the lower nicotine level, with a developing period of 2 to 3 hours. At the higher nicotine level the concentration in any series of determinations should be kept within a range of ± 0.5 gram with the same developing period.

TABLE 6.—*Light transmission in range of nicotine concentrations conforming to Beer's law*

NICOTINE CONCENTRATION	LIGHT TRANSMISSION
micrograms per ml.	*per cent*
0	100
5	81.2
10	66.5
20	43.9
30	29.3
40	20.4
50	13.3
60	9.2
70	6.4
80	4.3

The results for sodium sulfate are shown in Table 5. The concentration of this salt affects the transmission to an intermediate degree compared with the other salts. There is a small but definite rise in transmission values with increase in concentration of salt. The salt concentration should therefore be kept fairly uniform in any series of determinations. This salt also desensitizes the reaction. The optimum developing period is 2 hours, except at and below a concentration of 0.2 gram, where it is 3 hours.

Range of nicotine concentrations conforming to Beer's law.—It is known that the color reaction used here follows Beer's law, although no data are available to show the extent of the range conforming to this law. In Table 6 are given data showing applicability up to a concentration of at least 80 micrograms per ml. Beyond this concentration the color was too deep, and the precision of the photometer at the low end of the scale was inadequate for satisfactory tests.

SUMMARY

Data are presented on the nicotine color reaction produced by cyanogen bromide and β-naphthylamine, with the following findings:

(1) The optimum *p*H for maximum sensitivity is about 10. In the presence of excess alkali or acid, neutralization to the phenolphthalein end point is a practical means of adjusting a solution for the test.

(2) If an alkaline solution is to be prepared for the test, the sensitivity attained by neutralizing is in the following descending order of acids: acetic, hydrochloric, and sulfuric. When hydrochloric acid is used the sensitivity is practically equal to that in the absence of any salt.

(3) The optimum developing period in the presence of sodium acetate is 45 minutes in concentrations of the salt from 0.25 to 8.0 gram per 100 ml. In lower concentrations the period approaches $2\frac{1}{2}$ hours as the concentration decreases. Sodium acetate markedly sensitizes the reaction. Maximum sensitivity is attained at a salt concentration of 0.2 gram per 100 ml. with a developing period of 1 hour.

Sodium chloride exerts a slightly desensitizing effect on the development of color. The concentration may vary within ± 0.5 gram per 100 ml. The optimum developing period is 2–3 hours.

Sodium sulfate desensitizes the reaction more than does the chloride. The color diminishes fairly regularly with increasing concentration of salt; therefore, the latter should be kept uniform in a series of determinations. The optimum developing period is 2 hours, except below a salt concentration of 0.2 gram per 100 ml., when it is 3 hours.

(4) The color reaction follows Beer's law at least to 80 micrograms of nicotine per ml.

TURBIDIMETRIC DETERMINATION OF NICOTINE AS PHOSPHOTUNGSTATE

By L. N. MARKWOOD (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

Quantitative turbidimetric procedures can be used if reasonably stable suspensions of uniform colloidality can be attained. Such procedures, involving formation of the silicotungstate, have been applied to nicotine.

Kozu[1] described a method in which the nicotine silicotungstate precipitate was stabilized by means of starch. He also adopted the unique procedure of dissolving the precipitate that was first formed by warming, and reforming it by cooling. This technic made the test more sensitive. The degree of turbidity was measured in a nephelometer after the suspension had stood 20–30 minutes. Goodhue[2] developed a titration method, conducted in the presence of formic acid, which served to retard crystallization. He found it necessary also to add extract of Irish moss to prevent flocculation. Turbidity was followed photoelectrically, and the end point

[1] *J. Agr. Chem. Soc. Japan*, **7**, 977–83 (1931); *C. A.*, **26**, 2553 (1932).
[2] *Ind. Eng. Chem., Anal. Ed.*, **10**, 52–54 (1938).

(maximum turbidity) was located graphically from data obtained by overtitration.

It is obvious from these descriptions that the use of silicotungstic acid as precipitant requires special stabilization of the resulting suspension, and it is doubtful whether any procedures are fully satisfactory in repressing the strong tendency of nicotine silicotungstate to crystallize and settle out. If, instead of this acid, phosphotungstic acid is used, the colloidal property is adequate without the use of any protecting colloid, and a greater degree of reproducibility follows. At the same time the sensitivity is increased.

Heiduschka and Wolf[3] studied the behavior of these two acids toward a number of alkaloids. For nicotine they reported a sensitivity of 1:350,000 with silicotungstic acid and 1:1,000,000 with phosphotungstic acid. The composition of the phosphotungstate was fairly constant; at low acid concentrations it corresponded to the formula $3C_{10}H_{14}N_2 \cdot P_2O_5 \cdot 24WO_3 \cdot xH_2O$. They suggested the application of these two acids in turbidimetry for small amounts of alkaloids, but they presented no technic.

In the present study a satisfactory procedure was worked out with phosphotungstic acid in conjunction with the Kozu technic of warming and cooling. It was further found advantageous to introduce a small definite quantity of sulfuric acid to make the test more sensitive. Without sulfuric acid no turbidity results in concentrations of nicotine lower than 3 micrograms per ml.; with this acid a positive test can be had at 1 microgram per ml. This concentration represents the lower limit of the test. The upper limit is 5–6 micrograms per ml., beyond which point the density of the suspension makes reproducibility uncertain. The narrowness of this range is a limitation of the method, but an unknown solution can easily be diluted so that it will fall within these concentrations. A range between 3 and 10 micrograms per ml. can be obtained if no sulfuric acid is used. This range was not fully investigated, however, as the writer's needs were better met by the 1–6 microgram range; moreover, the results seemed more reproducible in the presence of sulfuric acid.

The optimum amount of sulfuric acid was first determined. The reagents used were phosphotungstic acid ($P_2O_5 \cdot 24\ WO_3 \cdot 42\ H_2O$) in 10 per cent strength and sulfuric acid (1+5).

To each 10 ml. of solution, containing a fixed quantity of nicotine and variable quantities of sulfuric acid, 4 drops (0.2 ml.) of phosphotungstic acid solution were added. The transmission of the resulting suspension was measured in a photoelectric photometer in which a test tube $7 \times \frac{7}{8}$ inches was used as the absorption cell, through which the beam of light passed horizontally. The suspension was then cleared by warming (not boiling) and restored by cooling (shaking) in cold water, after which the transmission was measured again.

[3] *Schweiz. Apoth. Ztg.*, 58, 213–8; 229–33 (1920).

TABLE 1.—*Optimum quantity of H_2SO_4 in phosphotungstate test for nicotine*
(Nicotine concentration, 3 micrograms per ml.)

H_2SO_4 (1+5) IN 10 ML. OF SOLUTION	LIGHT TRANSMISSION*	
	AFTER ADDING PHOSPHOTUNGSTIC ACID	AFTER WARMING AND COOLING
ml.	*per cent*	*per cent*
0.0	87.1	64.2
.1	85.7	46.0
.2	77.1	37.7
.3	76.9	37.8
.4	76.2	37.5
.5	75.0	38.3

* Water blank, 100.0 per cent.

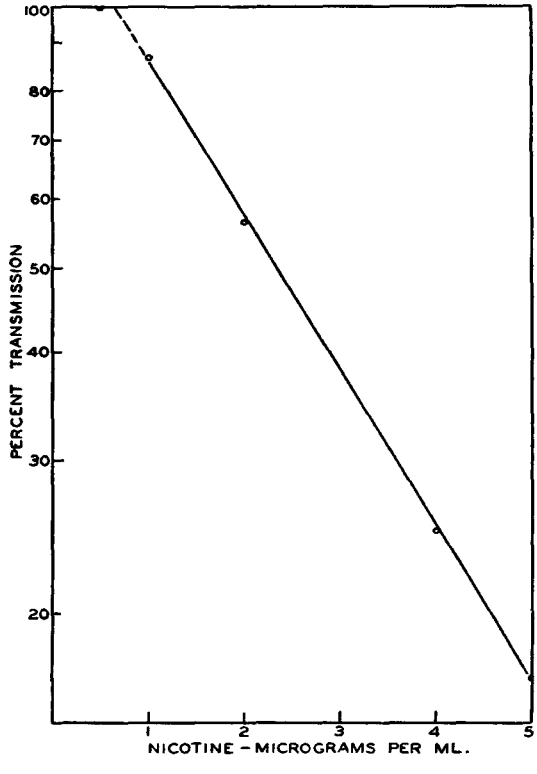

FIG. 1.—TYPICAL STANDARD CURVE FOR PHOSPHOTUNGSTATE DETERMINATION OF NICOTINE

The data in Table 1 show that sulfuric acid sensitizes the test and that the optimum amount is 0.2–0.4 ml. per 10 ml. of solution. A standard quantity of 0.2 ml. was selected for subsequent work.

To evaluate an unknown solution, a standard curve is prepared from known nicotine solutions by plotting concentrations against logarithms of photometer readings. This curve is a straight line, indicating a behavior similar to that shown by solutions that follow Beer's law. Since the nicotine precipitate has a certain solubility, the transmission remains at 100 per cent up to about 0.5 microgram of nicotine per ml.

The technic follows that already described for determining the optimum quantity of sulfuric acid, except that with each standard or unknown solution of nicotine 0.2 ml. of sulfuric acid was used. The temperature of the cooling water (about room temperature) is not important as long as there is no change (1–2°) during a series of determinations. A uniform time of 1 minute was adopted for cooling. Readings were then made without delay as this is the moment of greatest turbidity. The suspensions are fairly stable over a short period, about 15 minutes, but with the denser suspensions there soon begins an upward drift in light transmission, indicative of particle aggregation. After about an hour the upper portion of the liquid shows signs of clearing.

A typical run is portrayed in Figure 1.

The reproducibility of photometer readings is illustrated by the following results at two concentrations of nicotine:

LIGHT TRANSMISSION	
AT 2 MICROGRAMS NICOTINE/ML.	AT 4 MICROGRAMS NICOTINE/ML.
per cent	*per cent*
54.4	24.5
54.8	24.4
53.9	24.7
54.4	24.5
54.4	24.3
54.4	24.4
54.6	
54.2	Av. 24.5
54.5	
Av. 54.5	

The uniformity in results is considered to be proof that controlled conditions can be made to yield consistent values. The secret of uniformity appears to lie in not shaking the tube too vigorously during cooling—only enough to cool contents in 1 minute; stronger agitation tends to induce aggregation and the reading will be too high.

In sensitivity the method described here approaches, but does not equal, that reached by the colorimetric method involving cyanogen bromide and

β-naphthylamine.[4] Whereas that method is applicable down to a few tenths of a microgram per ml., the phosphotungstate method is not applicable below about 1 microgram per ml. Even so, the disparity is not great, and the phosphotungstate method has the advantage of giving the result immediately, whereas a period up to 3 hours is required with the color method.

The solution to be tested should preferably contain only nicotine, as in the distillate from a nicotine distillation. Organic bases and alkaloids, proteins, and amino acids, as well as certain metals, such as lead, are precipitated with phosphotungstic acid, and should therefore be absent. Organic solvents, such as alcohol, acetone, and ether, exert a solvent action on the precipitate and must be absent. Certain inorganic salts, such as sodium sulfate and magnesium sulfate, may be present if the standard solutions also contain them in uniform quantities.

SUMMARY

A turbidimetric method for determining nicotine in concentrations from 1 to 6 micrograms per ml. is described. In this method the turbidity produced by phosphotungstic acid, which is added to the nicotine solution containing a fixed quantity of sulfuric acid, is measured photometrically. The time required is only a few minutes.

DETERMINATION OF NICOTINE IN FRESH TOBACCO LEAF

By L. N. MARKWOOD (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

The need for a quick, practical method for determining the nicotine content of tobacco leaves at maturity or at any stage during growth led to the present investigation. The prime object is to furnish the tobacco breeder means whereby he can select from an experimental plot desired plants, based on nicotine content, without harvesting all the plants, reserving the seed, and subsequently analyzing each after special preparation of the material. Such a method was evolved and tested on several types of *Nicotiana tabacum* L. and on one sample of *N. rustica* L.

The principle of the method is as follows: A small portion of the fresh leaf is treated with strong sulfuric acid to disintegrate the tissue and bring the nicotine into solution. The extract is diluted and shaken with litharge to precipitate protein and other organic substances, and to neutralize the excess sulfuric acid. The solids are filtered off. Powdered magnesium is added to precipitate all trace of lead, which must be removed. After a second filtration the solution is ready for the actual determination of the nicotine, which may be accomplished either by the phosphotungstate

[4] Markwood, *This Journal*, 22, 427 (1939).

turbidimetric procedure[1] or by the colorimetric method involving cyanogen bromide and β-naphthylamine, hereafter called the color test.[2]

EXPERIMENTAL PROCEDURE

The sample of tobacco leaf usually consisted of a disk 19 mm. in diameter, punched out with a cork borer. Larger or smaller disks may be taken, depending on the thickness of the leaf and on the nicotine content. If the leaf is thin and of low nicotine content, two or three such disks may be used as one sample. The disk was weighed with care to prevent loss of moisture. At the same time another sample, preferably of several disks, was taken for the determination of moisture (drying at 100° C. for 4 hours).

The sulfuric acid used for disintegrating the leaf consisted of 9 parts (by volume) of concentrated acid to 1 part of water. The concentrated acid was avoided, because it generates considerable heat when acting on the leaf, which might cause some loss of nicotine by oxidation. The volume of acid was standardized at 6 drops (0.25 gram) per disk, the practical minimum for completely wetting the sample. The disk was placed in a small beaker or, better, a porcelain crucible, the bottom surface of which had been roughened by fusion of sodium carbonate. After the acid had acted for about 2 minutes, the disk was tamped with a flattened glass rod, also roughened. In most cases this tamping readily pulped the tissue, but some tough tobaccos required special care to insure complete pulping.

In a 100 ml. volumetric flask (larger for high-nicotine plants) were placed about 1.2 grams of litharge and 30 ml. of water, and the mixture was shaken to form a suspension. To it was added the sulfuric acid extract of the leaf, and this mixture was shaken until flocculation or clotting was evident, which usually occurred in 2–3 minutes. It was then made to volume, mixed well, and filtered rapidly through a dry paper.

The chief purpose of the litharge is to remove protein from the solution, since protein will later interfere with the phosphotungstate test. (Its possible interference in the color test has not been studied.) Besides removing the various organic substances such as gum and plant acids, litharge also neutralizes and removes the sulfuric acid. The solution is then slightly alkaline to phenolphthalein because of the presence of lead hydroxide. This condition is desirable, as some nicotine is adsorbed from acid solution by filter paper, but not from neutral or alkaline solution.

It is important to shake the flask until clotting occurs, which indicates that the sulfuric acid has been completely neutralized. The solids then readily settle out. The color of the insoluble matter is yellow, but of a lighter hue than the original litharge because of admixed lead sulfate. With pure nicotine solutions the color behavior is different, for after about 5 minutes' shaking the solid becomes completely white, owing to the formation of lead sulfate, and settles out readily; the acid is then completely neutralized and shaking may be stopped.

[1] Markwood, *This Journal*, 23, 800 (1940).
[2] *Ibid.*, 22, 427 (1939).

The filtrate is clear and colorless. It contains a small quantity of sulfate, due to the solubility of lead sulfate, which offers no interference. It also contains some lead, chiefly as lead hydroxide, which must be removed, as lead interferes both in the phosphotungstate test, by formation of insoluble lead phosphotungstate, and in the color test, where the alcohol precipitates lead sulfate. All trace of lead may be removed by shaking with about 0.25 gram of powdered magnesium. An equivalent amount of magnesium passes into the solution, which becomes saturated with magnesium hydroxide, but magnesium does not interfere in the subsequent tests. About 1 minute's shaking was sufficient to throw out the lead. The solution was then filtered again through a dry paper. It was the usual practice at this point to add 1 drop of phenolphthalein indicator and neutralize with 1 drop of very dilute sulfuric acid. This was not necessary for the phosphotungstate test, since sulfuric acid was to be added, but it was thought desirable to have the solution neutral for the color test. The dilution of the solution by 2 drops of liquid was immaterial. Aliquots of the final solution were taken for the nicotine determination, 10 ml. for the phosphotungstate test, and 5 ml. for the color test.

Subsequent study[3] has shown that it is not necessary to neutralize the solution at this point even for the color reaction, since the optimum *p*H for the reaction is 10 and the observed *p*H of the final magnesium hydroxide-saturated solution is 10.6. Standard solutions developed as much color without neutralization as with it.

The technic of determining small quantities of nicotine turbidimetrically as phosphotungstate was followed as described.[1] The color test was also followed as described,[2] except that the photometric readings were made after 3 hours, to allow time for development of maximum color. The phosphotungstate test has the advantage that it gives the result in a short time, but if the suspension is too dense it is necessary to repeat the test on a diluted solution.

In developing the method a somewhat different procedure was followed in the initial stages, for the purpose of establishing a control. The validity of the method rests upon finding the same quantity of nicotine by the procedure described as by steam distillation. The comparison can not be made simply on two adjoining disks, since nicotine distribution varies widely over the leaf. Therefore, the plan was adopted of treating a number of disks as a unit and analyzing aliquots of the solution. Usually 10 disks were treated with 60 drops of sulfuric acid, the extract was made to exactly 100 ml., and from this volume, after clarification by centrifuging, were drawn aliquots of 10 ml., each of which corresponded to one disk. The distillations were conducted in the presence of a slight excess of sodium hydroxide. The volume collected (100 ml.) was the same as the final volume of the corresponding litharge-treated aliquot. The final runnings

[3] Markwood, *This Journal*, 23, 792 (1940).

were always tested with phosphotungstic acid to confirm absence of nicotine. The residue of the distillation was also submitted to this test, after a litharge-magnesium purification.

RESULTS

The results obtained from several samples both by the proposed method and by distillation are summarized in Table 1.

TABLE 1.—*Nicotine content of several types of tobacco as determined by several methods*

SAMPLE		NICOTINE FOUND BY DISTILLATION		NICOTINE FOUND BY LITHARGE METHOD	
		PHOSPHOTUNGSTATE TEST	COLOR TEST	PHOSPHOTUNGSTATE TEST	COLOR TEST
		*per cent**		*per cent**	
High-nicotine tobaccos *Nicotiana tabacum:*					
Texas Cuban:					
Leaf A:					
Base		6.91	7.42	7.04	—
		7.04	7.27	7.18	—
	Av.	6.98	7.35	7.11	—
Tip		7.22	7.36	7.42	7.32
		7.32	7.42	7.32	7.13
	Av.	7.27	7.39	7.37	7.23
Leaf B, tip:					
Sample 1		9.37	9.63	9.20	9.27
		9.37	9.56	9.36	9.36
	Av.	9.37	9.60	9.28	9.32
Sample 2		7.28	7.12	7.28	7.40
		7.40	7.12	7.12	7.28
	Av.	7.36	7.12	7.20	7.36
Burley J		4.48	4.57	4.91	4.82
		4.33	4.62	4.91	4.86
	Av.	4.41	4.60	4.91	4.84
Nicotiana rustica:		5.90	5.99	5.77	5.77
		6.02	5.99	5.83	5.73
	Av.	5.96	5.99	5.80	5.75
Low-nicotine tobaccos *Nicotiana tabacum:*					
German		0.91	1.15	1.17	1.31
		.89	1.17	1.15	1.38
	Av.	.90	1.16	1.16	1.35
		micrograms per disk		*micrograms per disk*	
Maryland		160	160	640	240
		150	140	620	240
	Av.	155	150	630	240

* Dry basis.

Some of these values may appear too high in the light of previous data, but it should be remembered that conventional analyses are usually made on the whole leaf, including the midrib, which contains much less nicotine than the leaf tissue, and on the dried material, which has undoubtedly lost some nicotine in drying. It should also be noted that the present method operates on a small part of the total leaf area, certain parts of which are definitely richer in nicotine than others, whereas the dried sample gives the average content of the entire leaf.

Except in the low-nicotine tobaccos, there is no significant difference in the results between the phosphotungstate and the color tests. There is also satisfactory agreement by either of these tests between the distilled and the litharge-treated aliquots. The method is therefore proved valid for the high-nicotine Texas Cuban and Burley J types of *Nicotiana tabacum* and for *N. rustica.*

On a low-nicotine Maryland tobacco (*N. tabacum*), however, a marked difference between the two methods was shown. The values found by distillation are in agreement and represent the true nicotine content. The values found by the litharge method are appreciably higher, about 4 times higher than those by the phosphotungstate test, and 1.6 times by the color test. This discrepancy pointed to the presence in considerable amount of at least one other alkaloid, which was non-volatile with steam and was responsive to the phosphotungstate test but less so to the color test. A test of the distillation residue was strongly positive, showing the alkaloid to be relatively nonvolatile. It is, of course, obvious that the method should fail when other alkaloids are present, since it determines the total alkaloid content of the plant.

IDENTIFICATION OF ALKALOID

To identify the extraneous alkaloid four whole leaves were processed. They were first steam-distilled from slightly alkaline solution, whereby nicotine and some of the other alkaloid passed over. The latter, suspected of being a secondary base, was removed from the distilled fraction with p-toluenesulfonyl chloride. The picrate of the purified base melted at 223° C. (corr.), confirming it as nicotine dipicrate. The alkaline residue, containing most of the nonvolatile alkaloid, was purified by a litharge treatment, made strongly alkaline, and exhaustively extracted with ether. The compound obtained on evaporation of the ether was purified by fractional aqueous distillation and converted to the picrate, which melted at 188.5°–190° C. The compound therefore appeared to be nornicotine, a secondary base that is much less volatile with steam than is nicotine.[4] A mixed melting-point determination with l-nornicotine dipicrate furnished by C. R. Smith showed no change. Confirmation was furnished by methylating the alkaloid with formaldehyde and formic acid.[5]

[4] Smith, C. R., *J. Econ. Ent.*, 30, 724 (1937).
[5] Späth and Zajic, *Ber.* 68, 1667 (1935).

The picrate of the methylated product agreed in melting point with that of nicotine.

RELATIVE QUANTITIES OF NICOTINE AND NORNICOTINE

To obtain some idea of the relative amounts of nicotine and nornicotine in the Maryland tobacco, some of the dry material available from a previous crop was examined by the method described by Koenig.[6] Nicotine only is said to be removed by a magnesium oxide distillation, whereas both alkaloids are distilled off in the presence of sodium hydroxide. This method of separation is not perfectly sharp, and it favors a high result for nicotine. From the weights of the picrates obtained, this tobacco contained about 0.73 per cent of total alkaloids, of which about 95 per cent was nornicotine and 5 per cent was nicotine. That the total alkaloids consisted almost entirely of nornicotine was shown also by the melting point of the picrate of the unseparated alkaloid mixture (187–9°), which is practically the melting point of pure nornicotine.

The discovery of a strain of *Nicotiana tabacum* so rich in nornicotine was unexpected. The work of Pictet and Rotschy[7] apparently shows that the non-nicotine portion of tobacco alkaloids is small, approximately 2.5 per cent of the total. This order of magnitude probably holds for most commercial types of tobacco, but new types, especially of low nicotine content, are being cultivated and it may be necessary to revise the present conception of the composition of these alkaloids. Koenig[6] has called attention to the presence of appreciable quantities of nornicotine in some tobaccos, especially the low-nicotine types. Further intimations along this line are contained in a paper by Koenig and Dörr,[8] who suggest, but do not name, another alkaloid present in significant quantity.

The present work is apparently the first finding of a preponderance of nornicotine in an American tobacco. So far as available evidence indicates, it is the low-nicotine plants that are likely to harbor nornicotine. There is therefore some plausibility to the idea that, when nicotine formation is repressed by selective breeding, nature tends to compensate by creating other alkaloids.

It may become necessary in the future to make determinations of both alkaloids in order to evaluate a tobacco or tobacco blend. The effect of nornicotine on quality seems not to have been considered. At the present time fully satisfactory separations of these alkaloids are not known, especially where small quantities are involved. Lack of a supply of pure nornicotine prevented a study of its chemical behavior and the possibility of adapting the method to this type of tobacco, but the writer intends to prepare some of this alkaloid in the near future. Although the method is not applicable here, it is worth noting that the color test, with a value only

[6] Handbuch der Lebensmittel-Chemie, VI Band, Berlin, 1934, p. 296.
[7] *Compt. rend.*, 132, 971 (1901).
[8] *Z. Untersuch. Lebensm.*, 67, 113 (1934).

1.6 times too high, might be modified to block the chromogenic activity of this alkaloid entirely.

RESULTS FOR LOW-NICOTINE GERMAN TOBACCO

For the tobacco of German origin mentioned in Table 1, the color values are somewhat higher than the phosphotungstate values, and the values by the litharge method are higher than those by distillation. The inference, if any significance attaches to these differences, is that a relatively nonvolatile base is present to a small extent, and that a third volatile base is present, which is more responsive to the color test than to the phosphotungstate test. This conclusion is offered with some reserve, however, as more evidence is required. The method may be considered applicable to this tobacco for approximate results. Some dried material from a previous crop was examined by the method of Koenig.[8] Total alkaloids were found to amount to 0.20 per cent, of which 91 per cent could be accounted for as nicotine and 9 per cent as nornicotine.

An idea of the possible usefulness of the present method may be gained from the study of the distribution of nicotine in the tobacco plant by Andreadis and Toole.[9] Following the conventional procedure of drying the plant, distilling off the nicotine and determining the latter as silicotungstate, they found it necessary by this method to use 200 leaves, from which composite samples of corresponding areas were prepared. By the method presented here it is possible to prepare a nicotine-distribution chart from one leaf.

SUMMARY

A short method is described for determining the nicotine content of fresh tobacco. No distillation is involved, and the method is adapted to large-scale routine tests, especially those used in plant breeding. It consists essentially in digesting a small area of the leaf with sulfuric acid, treating the resulting solution with litharge for purposes of removing protein and neutralizing, treating with magnesium for removal of lead, and then measuring the nicotine either turbidimetrically as phosphotungstate or colorimetrically with cyanogen bromide and β-naphthylamine.

The data presented show the applicability of the method to several types of *Nicotiana tabacum* and to *N. rustica*. The method gives true results where nicotine is the only alkaloid. A low-nicotine Maryland tobacco, however, was found to contain about 95 per cent of the total alkaloids as nornicotine. In such cases abnormally high results are found.

ACKNOWLEDGMENT

This work was made possible through the cooperation of W. W. Garner and C. W. Bacon, of the Bureau of Plant Industry, U. S. Department of Agriculture.

[8] *Z. Untersuch. Lebensm.*, 77, 262 (1939).

THE DETECTION AND DETERMINATION OF DRIED SKIM MILK IN MEAT PRODUCTS

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Within the past 20 years, the use of dried skim milk as an ingredient of meat products has become commonplace. It is used in small to moderate quantities as a constituent of many brands of sausage, loaves, and other chopped or comminuted meat food products.

The regulations governing the meat inspection of the United States Department of Agriculture limit the quantity of dried skim milk in sausage to 3.5 per cent of the finished product, and require marking, branding, or labeling of the product to show its presence. The increasing use of this material as a constituent of comminuted meat food products and the limitation of the quantity permitted in sausage have made better analytical methods for its detection desirable and even imperative. This paper outlines several tests and procedures that have been found to be of value and discusses some of the difficulties and problems that arise in the qualitative or quantitative determination of dried skim milk in comminuted meat products.

Dried skim milk is the product resulting from the removal of water from skim milk. That from the manufacturing processes in use at the present time is a white to a very slightly yellowish powder with a characteristic and rather pleasant odor and taste. Its normal composition is approximately 5 per cent moisture, 35–36 per cent protein (N×6.25), and 50–52 per cent lactose ($C_{12}H_{22}O_{11} \cdot H_2O$). The calcium content, calculated as calcium oxide, is 1.72–1.97 per cent, and the total ash varies from 7.8 to 8.5 per cent.

Neither meat nor meat products normally contain lactose, so the lactose added in the form of dried skim milk provides a convenient basis for its detection and determination. Also, since dried skim milk contains from 75 to 80 times more calcium than does meat, the determination of calcium in comminuted meat products will, in the absence of interference due to materials containing that substance, serve as a means of detecting and estimating the quantity of dried skim milk present.

Another method was proposed by Jacobs,[1] and a similar one by Schneck and Ziegler.[2] Both are based on the separation and determination of casein, which does not occur in meat, but which has been found to comprise approximately three-fourths of the protein material in dried skim milk. However, this procedure seems to be only approximately quantitative, and would appear to be of value chiefly as a qualitative method.

[1] Chemical Analysis of Foods and Food Products (1938), p. 203.
[2] *Vorratspflege Lebensm. Forsch.*, 1, 494 (1938).

DETECTION OF LACTOSE BY THE FORMATION OF LACTOSAZONE

Lactose, the principal constituent of dried skim milk, reacts with phenylhydrazine to form lactosazone, which is easily recognizable under the microscope by its characteristic crystalline structure and the identification of this osazone has been used as a test for lactose. Cole[3] developed a procedure based on this reaction for the detection of lactose in urine, and it has been adapted in this laboratory to serve as a means of detecting dried skim milk in sausage and comminuted meat products.[4]

The following procedure is based on the fact that activated charcoal adsorbs the lactose from an aqueous extract of the meat product. The lactose is then removed from the charcoal by treatment with a small volume of dilute acetic acid, a procedure that permits concentration of the sugar into a small volume of filtrate and its separation by filtration or centrifuging from most of the interfering protein material. When so prepared, lactosazone crystallizes in characteristic "hedgehog" clumps with projecting spines, which usually, although not always, terminate in long hair-like appendages. Recrystallization is generally not satisfactory, as the resulting crystals are much smaller than those originally formed and are usually less characteristic. Photographs and drawings showing the crystalline form of lactosazone as well as osazones of the other common sugars are to be found in many standard texts on physiological chemistry and microscopy, but the observer should become familiar with the crystalline forms obtained under the specific conditions of the procedure used.

PROCEDURE

To 25 grams of finely divided meat or product in a 250 ml. beaker add 1–2 grams of powdered $CaCO_3$ and 50 ml. of warm water. Thoroughly break up any lumps with a glass rod, and boil the mixture a few minutes. Centrifuge, and separate the fat from the aqueous solution with the aid of a separatory funnel. (The aqueous solution may also be obtained by filtering the boiled mixture through a wet folded filter paper.) To 25–30 ml. of the aqueous solution add 10 ml. of alumina cream, shake, allow to stand a few minutes, and filter through a wet filter paper. Add 25 ml. of the filtrate to 1 gram of good adsorbent charcoal in a 125 ml. Erlenmeyer flask, shake vigorously, boil a few seconds, cool thoroughly, and allow to stand 10 minutes, with frequent shaking. Filter with suction through a Gooch crucible containing a disk of filter paper. Wash with 2–3 ml. of water and suck dry. Transfer the charcoal from the crucible to a 125 ml. Erlenmeyer flask, add 10 ml. of water and 1 ml. of glacial acetic acid, and boil for about 10 seconds. Filter the hot mixture through a small filter paper into a large test tube containing 0.5 gram of pure phenylhydrazine hydrochloride and 2 grams of sodium acetate. Place tube in a boiling-water bath, and, after a few minutes, mix by shaking, and heat 45 minutes. Remove the tube from the bath, filter hot, and allow to stand at room temperature at least 1 hour. If no crystals have formed after 1 hour, stopper tube and allow to stand until crystals form. The test may be considered negative if no lactosazone crystals form after standing 18 hours. Pipet off a little of the deposit, if any, onto a

[3] Practical Physiological Chemistry, 9th Ed. (1933), p. 318.
[4] *This Journal*, 19, 410 (1936).

microscope slide, cover with a cover-glass, and examine microscopically with a magnification of 100–150 diameters.

ESTIMATION OF DRIED SKIM MILK BY DETERMINATION OF CALCIUM

A number of analyses of meat and milk products made in this laboratory show that dried skim milk contains 75–80 times more calcium than do

TABLE 1.—*Analyses of some commercial grades of dried skim milk*

SAMPLE NO.	ASH	N	CaO
	per cent	*per cent*	*per cent*
1	8.1	5.95	1.86
2	8.3	5.68	1.77
3	8.4	5.72	1.97
4	8.1	5.81	1.84
5	8.4	5.52	1.84
6	7.8	5.98	1.84
7	8.2	5.54	1.72
8	7.8	5.93	1.89
9	7.9	5.73	1.81
10	8.1	5.98	1.89
11	7.8	5.46	1.74
12	8.3	5.92	1.84
13	8.5	6.05	1.89
14	8.2	5.91	1.84
15	8.3	5.94	1.85
Maximum	8.5	6.05	1.97
Minimum	7.8	5.46	1.72
Average	8.1	5.81	1.84

TABLE 2.—*Analyses of some meats and animal products known to be free from dried skim milk*

PRODUCT	ASH	N	CaO
	per cent	*per cent*	*per cent*
Lean beef (salted)	1.39	3.30	0.013
Smoked ham	4.76	2.73	0.034
Smoked ham	3.16	2.60	0.031
Cooked pork skins	0.66	3.68	0.010
Scalded beef liver	—	4.85	0.013
Beef trimmings	0.78	3.20	0.012
Cracklings	4.10	—	0.25
Cracklings	6.50	—	0.14
Cracklings	6.70	—	0.10
Cracklings	5.8	—	0.12

meat or products used in making sausage. On this basis, it can be calculated that the presence of 3.5 per cent of dried skim milk in a sausage product would produce a 4- to 5-fold increase in the calcium content of the ash. This is shown in Tables 1–5, which also show the calcium content of certain other substances often mixed with meat products.

The large number of analyses made (represented in Tables 1–5) show that the calcium oxide content of normal meat products averages 0.024 ±0.008 per cent. Likewise, dried skim milk contains 1.84±0.06 per cent calcium oxide. So with due regard for the normal fluctuations in the

TABLE 3.—*Analyses of a number of sausage and comminuted meat products that did not contain dried skim milk*

PRODUCT AS LABELED	ASH	N	CaO
	per cent	*per cent*	*per cent*
Fresh sausage	3.26	2.04	0.025
Fresh sausage	2.60	1.62	0.025
Fresh pork sausage	2.02	1.45	0.024
Fresh pork sausage	2.58	1.73	0.019
Smoked sausage	3.78	2.32	0.021
Smoked link sausage	3.58	2.45	0.022
Luncheon meat	3.56	2.35	0.019
Luncheon meat	3.48	2.53	0.018
Luncheon style sausage	3.40	2.05	0.023
Frankfurter style sausage	2.64	2.59	0.024
Frankfurter style sausage	3.28	1.92	0.028
Frankfurter style sausage	3.37	2.41	0.031
Bologna style sausage	4.22	2.24	0.017
Bologna style sausage, all beef	3.15	2.65	0.026
Bologna style sausage	3.35	1.92	0.019
Bologna style sausage	3.20	2.12	0.032
Bologna style sausage	3.44	2.28	0.030
Average			0.024

TABLE 4.—*Ash and calcium content of some materials commonly used in the manufacture of comminuted meat products*

PRODUCT	ASH	CaO
	per cent	*per cent*
Wheat flour	0.32	0.03
Corn flour	0.63	0.009
Corn flour	0.32	0.005
Corn flour	0.62	0.009
Soybean flour (Nusoy)	2.9	0.60
Soybean flour	5.8	0.41
Soybean flour	6.2	0.40
Soybean flour	5.8	0.28

calcium oxide content of both meat and dried skim milk, the percentage of calcium oxide in the meat product can, in the absence of interfering substances, be used as a measure of the quantity of dried skim milk present.

Owing to the fluctuations in the calcium oxide content of meat and dried skim milk, the procedure presented here will not detect with cer-

tainty a quantity of dried skim milk below 0.5 per cent, but if the amount is greater than 1 per cent, it can usually be determined within ± 0.5 per cent. Since this method is based on the increased calcium oxide content of the ash, it is evident that the method will become unreliable if substances or ingredients that contain appreciably more calcium than meat are used in significant quantities. The analyses of numerous samples of meat and meat by-products as well as substances often used in their preparation, collected from the territory adjacent to the Atlantic coast, failed to disclose any material having a calcium oxide content sufficiently high to invalidate the method. However, it was later discovered that beef tripe, a product that may be, and oftentimes is, used in certain kinds of sausage

TABLE 5.—*Analyses of sausage and comminuted meat products known to contain dried skim milk*

PRODUCT	ASH	N	CaO	DRIED SKIM MILK CALCULATED FROM THE CaO
	per cent	*per cent*	*per cent*	*per cent*
Comminuted meat mixture, 2.5% dried skim milk added	3.21	2.94	0.070	2.5
Comminuted meat mixture, 5% soybean flour and 1% dried skim milk added	3.20	3.15	0.050	1.4
Comminuted meat mixture, 5% corn flour and 3% dried skim milk added	3.13	2.94	0.080	3.0
Comminuted meat mixture, 5% soybean flour and 3% dried skim milk added	3.32	3.20	0.089	3.5
Frankfurt style sausage, dried skim milk added	3.33	1.89	0.086	3.4
Frankfurt style sausage, dried skim milk added	3.43	2.63	0.096	3.9
Smoked link sausage, dried skim milk added	2.36	2.43	0.085	3.3
Knockwurst, dried skim milk added	3.30	2.43	0.104	4.3

may sometimes show an abnormally high content of calcium oxide. This is particularly true of tripe originating in certain sections of the Middle West, in regions near the Missouri River. Further investigation indicated that this condition was brought about during the cleaning of the tripe by the action of hot alkaline solutions on the hard waters prevalent in the regions where the tripe is prepared. A precipitate of calcium salts was thus deposited so deeply within the tissues of the tripe that it was not subsequently removed. The calcium oxide content of a number of samples of beef tripe collected from various localities throughout the United States is shown in Table 6.

Although tripe is usually a minor constituent of comminuted meat products, instances have been observed in which the amount of calcium oxide introduced by this means was sufficient to impair the accuracy of the method. However, for products in which no beef tripe has been

used, or for products known not to contain beef tripe produced in those areas having waters of high mineral content, the determination of calcium by the following procedure has been found both convenient and reliable for estimating dried skim milk.

TABLE 6.—*Calcium oxide content of samples of beef tripe collected from various places throughout the United States*

SAMPLE COLLECTED AT—	CALCIUM OXIDE FOUND
	per cent
Chicago, Ill.	0.011
Dothan, Ala.	0.015
	0.015
Salem, Va.	0.018
Buffalo, N. Y.	0.022
Baltimore, Md.	0.027
	0.016
	0.029
	0.027
Atlanta, Ga.	0.028
	0.023
Memphis, Tenn.	0.036
Cleveland, Ohio	0.039
Jacksonville, Fla.	0.036
	0.044
	0.040
Austin, Minn.	0.037
	0.054
Nashville, Tenn.	0.049
South St. Paul, Minn.	0.042
	0.050
	0.029
South St. Joseph, Mo.	0.051
	0.060
	0.043
Omaha, Nebr.	0.058
Ottumwa, Iowa	0.059
Mason City, Iowa	0.083
Waterloo, Iowa	0.085
Sioux City, Iowa	0.087
	0.130
	0.069
	0.099
	0.150

PROCEDURE

Press firmly against the sides and bottom of a tared porcelain or platinum dish, approximately 25 grams of the finely ground and well mixed sample. Reweigh at once to the nearest 0.1 gram. Place the dish in an unheated electric muffle furnace and slowly increase the heat, allowing free access of air. As soon as the contents of the dish have ignited, open the door of the muffle and allow all combustible material to burn away; close the muffle, increase the heat to 500°–600° C., and com-

plete the ashing at this temperature. The ash need not be pure white, as the presence of a few particles of unburned carbon is not an appreciable source of error.

Add 5 ml. of concentrated HCl to the ash and evaporate to dryness on the steam bath. Dissolve the residue in 15–20 ml. of warm HCl (1+4), and filter into a 125 ml. Erlenmeyer flask. Wash the paper and residue thoroughly with warm water, using about 30 ml. in all, and add the washings to the original filtrate. Add 10 ml. of saturated $(NH_4)_2C_2O_4 \cdot H_2O$ solution, and heat to boiling. Remove from the heat, add 1–2 drops of methyl red indicator, and, with constant shaking, add NH_4OH (1+1) from a buret until a permanent turbidity appears; or if no turbidity appears, until the solution is neutral to methyl red. Boil the solution for a few minutes and if not already neutral, complete the neutralization with the dilute NH_4OH, and allow to stand for several hours. If the original material contained much dried skim milk, a precipitate of calcium oxalate will appear and remain while the solution is still distinctly acid to methyl red; if no dried skim milk was present, the turbidity due to calcium oxalate may not appear until the neutral point has been nearly or completely reached.

Filter and wash with water at room temperature until the filtrate is free from oxalates. Then pierce the tip of the filter paper with a platinum wire and with warm water wash the precipitate into the flask in which the calcium was originally precipitated. Add about 10 ml. of H_2SO_4 (1+4) and sufficient water to make the total volume about 60 ml. Heat to about 90° C. and titrate to a permanent pink color with 0.05 *N* $KMnO_4$, then add the filter paper to the solution and complete the titration. Standardize the $KMnO_4$ solution against $Na_2C_2O_4$ and calculate the Ca as CaO.

$$\frac{\%\ \text{CaO} - 0.024}{0.0184} = \%\ \text{dried skim milk.}$$

DETERMINATION OF LACTOSE BY SELECTIVE FERMENTATION

This quantitative method is based upon the determination of lactose after the other common reducing sugars have been removed by a treatment with yeast. Somogyi,[5] in his studies on the determination of blood sugars, found that as much as 800 mg. of dextrose per 100 ml. of blood could be removed within 5 minutes by treatment with yeast. Raymond and Blanco[6] confirmed the work of Somogyi, and found that under the same conditions, mannose, levulose, and sucrose were also removed, although not as rapidly as dextrose. Lactose, galactose, maltose, ribose, xylose, and arabinose were among those sugars found not to be affected by the yeast.

The results of Somogyi and of Raymond and Blanco have also been confirmed by work carried out in this laboratory. It was found that 5 ml. of a washed yeast suspension would, when shaken with 35–40 ml. of dextrose solution, immediately absorb as much as 10 mg. of dextrose and remove it quantitatively from solution when centrifuged. Table 7 shows the rate of removal of dextrose at room temperature from solutions containing 100 mg. of dextrose.

Under these conditions 100 mg. of dextrose is equivalent to approxi-

[5] *J. Biol. Chem.*, 75, 33 (1927).
[6] *Ibid.*, 79, 649 (1928).

mately 3 per cent in the original meat product, and it will be noted that removal is complete in 45 minutes.

Bailey[7] developed a method of this type for the determination of dried skim milk in sausage. The method proposed here differs from the Bailey method in that a simplified extraction is used, and a different and more rapid procedure is utilized for the determination of the remaining reducing sugars.

Fresh meat normally contains a small quantity of dextrose. During curing operations, and as a seasoning, a meat product may have mixed with it small quantities of sucrose, dextrose, and invert sugar. Lactose may be added in the form of dried skim milk. By subjecting the aqueous extract of a meat product to a fermentation by yeast, the reducing sugars, dextrose, and levulose, as well as the non-reducing sugar, sucrose, are re-

TABLE 7.—*Effect of time on the removal of dextrose by yeast*

TIME OF STANDING WITH THE YEAST	DEXTROSE REMAINING	DEXTROSE REMOVED
minutes	*mg.*	*per cent*
1	90	10
6	78	22
11	69	31
15	49	51
16	48	52
30	6	94
45	0	100
60	0	100

moved. Of the sugars that are not affected by this treatment, lactose and maltose are the only ones sufficiently common to warrant consideration as constituents of meat products. Even maltose, although used extensively in many food products and beverages, is not known to be a constituent of any of the substances customarily used in the preparation of meat products. Therefore the sugar remaining in the clarified aqueous extract of a meat product, after treatment with yeast, may be considered to be lactose.

In the proposed procedure, the finely comminuted meat product is warmed with water for approximately 30 minutes, and then cooled, acidified, treated with phosphotungstic acid, and made up to volume with water, and a correction is made for the volume occupied by the meat and precipitate. After vigorous shaking, the mixture is filtered, and an aliquot of the filtrate is neutralized and again made up to volume. A portion of the neutralized solution is treated with washed yeast for a period of one hour to remove any fermentable sugars. The yeast must be free from any colloidal or water-soluble constituents, so that after the treatment is complete, the yeast may be quantitatively removed by centrifuging. After centrifuging and decanting from the yeast, the lactose is determined in the

[7] Connecticut Agr. Expt. Sta. Bull. 401 (1937), p. 869; *Ibid.*, 415 (1938), p. 415.

clear liquid by a modification of the Scales method for reducing sugars.[8] The quantity of dried skim milk present is considered to be twice the quantity of lactose found.

In attempting to recover measured quantities of lactose from meat by this method, it was found that, while amounts of lactose larger than 0.5 per cent could be recovered quantitatively, low results were always obtained when less than 0.5 per cent lactose was present. It appeared that unless a definite minimum quantity of the reducing sugar was present, complete reduction of the sugar with the formation of cuprous oxide did

TABLE 8.—*Recovery of added quantities of dried skim milk*

LACTOSE ADDED TO MEAT (%)	LACTOSE ADDED TO ALIQUOT USED FOR REDUCTION (MG.)	VOLUME OF IODINE SOLUTION ADDED (ML.)	RETURN TITRATION WITH $Na_2S_2O_3$ (ML.)	VOLUME OF I_2 TO WHICH THE $Na_2S_2O_3$ IS EQUIVALENT (ML.)	VOLUME OF IODINE SOLUTION USED (ML.) (C—E)	VOLUME OF I_2 USED AFTER DEDUCTION FOR LACTOSE ADDED IN COLUMN B (ML.)	LACTOSE RECOVERED (%)	DIFFERENCE (%) (H—A)
A	B	C	D	E	F	G	H	I
0.0	10	10	4.06	3.76	6.42	0.04	–	–
0.15	10	10	3.09	2.77	7.23	0.82	0.15	0.00
0.25	10	15	7.06	7.19	7.81	1.39	0.27	0.02
0.35	10	15	7.37	6.61	8.39	1.09	0.37	0.02
0.45	10	15	5.88	6.00	9.00	2.58	0.50	0.05
0.70	0	10	6.85	6.14	3.86	3.86	0.72	0.02
0.80	0	10	5.71	5.82	4.18	4.18	0.82	0.02
1.20	0	10	3.48	3.54	6.46	6.46	1.26	0.06
1.40	0	10	3.04	2.72	7.28	7.28	1.37	0.03
1.60	0	15	7.18	7.42	7.58	7.58	1.50	0.10
2.00	0	15	4.83	4.92	10.08	10.08	1.96	0.04

not occur. This difficulty was circumvented, however, by repeating the determinations that did not show appreciable evidence of reduction at the end of the period of boiling and using another aliquot to which a measured quantity of lactose had been added. The amount of reduction due to the lactose present in the meat could then be calculated by deducting from the total reduction, the reduction due to the added lactose. With this procedure it was possible to recover added lactose from meat with the accuracy shown in Table 8.

The details of the method are as follows:

REAGENTS

(a) *Washed yeast suspension.*—Macerate two cakes of compressed yeast with about 150 ml. of water until a smooth suspension is obtained. Centrifuge for 5 minutes and discard the aqueous layer. Repeat the maceration with water and subsequent centrifuging four more times, or until the supernatant liquid after centrifuging is practically clear. Again suspend the yeast in water and make up to a

[8] Scales, F. M., *J. Biol. Chem.*, 23, 81 (1915); *J. Ind. Eng. Chem.*, 11, 747 (1919).

volume of 100 ml. Keep the yeast suspension in the refrigerator at approximately 4° C. and shake well before using. Discard after 2 weeks.

(b) *Benedict's solution.*—Dissolve 16 grams of $CuSO_4 \cdot 5H_2O$ in 125–150 ml. of water. Dissolve 150 grams of $Na_3C_6H_5O_7 \cdot 2H_2O$, 130 grams of Na_2CO_3 (anhydrous), and 10 grams of $NaHCO_3$ in about 650 ml. of hot water. Combine the two solutions cool, make up to 1 liter, and filter.

PROCEDURE

To 10 grams of the comminuted meat product in a 150 ml. beaker, add approximately 80 ml. of warm water, and break up all lumps with a stirring rod. Warm on the steam bath 30–60 minutes with occasional stirring, then transfer the mixture quantitatively to a 100 ml. volumetric flask, and cool in a bath of cold water. Add 2 ml. of concentrated HCl and 5 ml. of 20% phosphotungstic acid solution, make up to volume with water, and add 5 ml. of additional water to correct for the volume occupied by the meat and precipitate. Mix well and filter. Transfer 40 ml. of the filtrate to a 50 ml. volumetric flask, neutralize with NaOH solution, and make up to volume.

In a lipless 50 ml. centrifuge tube, place 5 ml. of the washed yeast suspension, and after centrifuging, drain off and discard the water. Add about 40 ml. of the above neutralized filtrate to the centrifuge tube containing the yeast mass, stopper the tube, and shake vigorously to dislodge and suspend the yeast. Allow to stand with occasional shaking for 1 hour. Then centrifuge again and determine lactose in an aliquot of the clear solution as follows:

Place 20 ml. of the Benedict solution in a 300 ml. Erlenmeyer flask and add 10 ml. of the above solution containing 5–25 mg. of lactose. If it is necessary to use less than 10 ml., add sufficient water to make 10 ml. Heat the solution to boiling, using a flame or electric hot plate that will bring the solution to a boil in 3–5 minutes. Continue the boiling for exactly 3 minutes, then cool quickly by holding the flask in a stream of cold water. If no appreciable reduction appears to have taken place at the end of the boiling period, discard the determination, and to another 10 ml. aliquot add 1 ml. of standard lactose solution (1 gram lactose in 100 ml.), and 20 ml. of the Benedict solution, and proceed as directed previously. Deduct from the total volume of iodine absorbed, the volume required to dissolve the Cu_2O from the reduction of 10 mg. lactose.

Dilute the cooled solution containing the reduced Cu_2O with 100 ml. of cold water, and from a pipet add slowly and with shaking, 10 ml. of acetic acid (240 ml. per liter). Then with constant shaking add a measured volume of iodine solution (approximately 0.04 *N*) at least 5 ml. in excess of that quantity necessary to dissolve the cuprous oxide and produce a perfectly clear, dark green solution. Allow to stand for a few minutes, then from a graduate add 20 ml. of phosphoric acid (240 ml. of the 85% ortho acid per liter), and titrate the excess iodine with thiosulfate solution (approximately 0.04 *N*), using starch solution as an indicator.

To determine the relationship between the iodine and thiosulfate solutions, mix 20 ml. of the Benedict solution and 10 ml. of water, and boil 3 minutes as directed previously. Cool, add 100 ml. of water, 10 ml. of the acetic acid, 10 ml. of the iodine solution, and 20 ml. of the phosphoric acid, and titrate the excess iodine with the thiosulfate solution.

Determine the lactose factor for the iodine solution by a repetition of the above procedure, adding to the solution before boiling, 1 ml. of the lactose solution. Calculate the percentage of dried skim milk by the formula,

$$\% \text{ dried skim milk} = \frac{KV_x}{5A},$$

where K is the number of mgs. of lactose represented by each ml. of the iodine solu-

tion; V_s is the volume (ml.) of iodine solution absorbed, and A is the weight in grams of meat represented by the aliquot used for the determination. (When the aliquot taken is 10 ml., $A = 0.8$ gram.)

CONCLUSIONS

Dried skim milk in meat food products may be detected and determined through the identification and determination of lactose or calcium, both of which are present in nearly constant proportions.

Lactose can be identified with certainty by the preparation and identification of lactosazone, but no quantitative method for the determination of lactose through the separation of the lactosazone has been developed.

Lactose in meat products may be accurately determined by the selective fermentation method presented, which fails only in the presence of soluble reducing, non-fermentable sugars other than lactose.

In the absence of substances containing sufficient calcium to interfere, dried skim milk may also be estimated by a determination of calcium. Such an estimation is applicable even to those samples that are received in such bad condition that the lactose may have been partly or wholly destroyed by bacterial action.

SEPARATION AND ESTIMATION OF *p*-PHENYLENEDIAMINE IN MIXTURES

By JONAS CAROL (U. S. Food and Drug Administration, Chicago, Ill.)

p-Phenylenediamine, $H_2N \cdot C_6H_4 \cdot NH_2$, is widely used in hair dye preparations, frequently in combinations with other amine compounds.

In the course of an examination of hair dyes that contained *p*-phenylenediamine, *p*-aminophenol, and metol it was found that *p*-phenylenediamine was precipitated by silicotungstic acid, whereas the other two amines were not. An attempt to use this reaction to determine *p*-phenylenediamine quantitatively by the general procedure specified for the determination of nicotine[1] failed because the weight of the residue obtained by ignition of the precipitate was not constant nor did it correspond to any definite molecular combination of *p*-phenylenediamine and silicotungstic acid.

A further study of the reaction showed that the precipitate formed slowly and was appreciably soluble in water at room temperature. When the reaction mixture was allowed to stand overnight in a refrigerator, however, complete precipitation of the *p*-phenylenediamine compound, $[C_6H_4(NH_2)_2]_2 \cdot SiO_2 \cdot 12WoO_3 \cdot 3H_2O$, resulted. On ignition this precipitate gave a residue, $SiO_2 \cdot 12WoO_3$, which corresponded definitely to the weight of *p*-phenylenediamine. The ratio of the weights of $2C_6H_4(NH_2)_2$ and $SiO_2 \cdot 12WoO_3$ was 0.0760.

[1] *Methods of Analysis, A.O.A.C.*, 1935, p. 60.

PROCEDURE

REAGENTS

Silicotungstic acid reagent.—Dissolve 120 grams of $SiO_2 \cdot 4H_2O \cdot 12WoO_3 \cdot 22H_2O$ in water and make to 1 liter.

DETERMINATION

To 50 ml. of an aqueous solution containing 0.02–0.05 gram of *p*-phenylenediamine, neutral to methyl red, add 25 ml. of the silicotungstic acid reagent. Stir until precipitation takes place. Place in a refrigerator and let stand overnight. Filter into a weighed Gooch crucible, transferring as much of the precipitate as possible. Transfer the remainder of the precipitate to the crucible with the aid of the filtrate. Wash the precipitate with two 5 ml. portions of ice water. Dry, ignite at 500° C. until all organic matter is destroyed, cool, and weigh. Weight of residue $\times 0.0760$ = Weight of *p*-phenylenediamine.

Solutions containing known quantities of *p*-phenylenediamine, both alone and in mixtures, were analyzed by this method. Tables 1 and 2 show the results.

TABLE 1.—*p-Phenylenediamine alone*

QUANTITY ADDED	WT. OF PRECIPITATE DRIED AT 110° C.	RECOVERY*	WT. OF RESIDUE ASHED AT 500° C.	RECOVERY†
gram	*grams*	*per cent*	*grams*	*per cent*
0.1002	1.4322	99.21	1.3077	99.19
0.1070	1.5323	99.40	1.3985	99.33
0.1035	1.4830	99.46	1.3543	99.45
0.1060	1.5243	99.80	1.3910	99.74

* Factor: $\frac{2C_6H_4(NH_2)_2}{[C_6H_4(NH_2)_2]_2SiO_2 \cdot 12WoO_3 \cdot 3H_2O} = 0.06941.$

† Factor: $\frac{2C_6H_4(NH_2)_2}{SiO_2 \cdot 12WoO_3} = 0.0760.$

TABLE 2.—*p-Phenylenediamine in mixtures**

QUANTITY ADDED	WT. OF PRECIPITATE DRIED AT 110° C.	RECOVERY	WT. OF RESIDUE ASHED AT 500° C.	RECOVERY
gram	*gram*	*per cent*	*gram*	*per cent*
0.0429	0.6129	99.16	0.5620	99.56
0.0429	0.6136	99.28	0.5629	99.72
0.0429	0.6139	99.33	0.5624	99.63
0.0429	0.6126	99.12	0.5600	99.21

* In addition to 0.0429 gram of *p*-phenylenediamine each of these mixtures contained 0.1 gram of metol 0.1 gram of *p*-aminophenol hydrochloride, and 0.1 gram of sodium sulfite.

Two mixtures, A and B, were prepared and submitted to collaborators for analysis by the proposed method. The compositions of the mixtures and the results reported by the collaborators appear in Table 3.

The close agreement of the collaborators' results indicates the accuracy of the method. The procedure is easily carried out and requires little actual working time. No extractions are needed; the determination can be made

directly upon a solution without first separating such interfering substances as aminophenols.

TABLE 3.—*Collaborative results*

COLLABORATOR	MIXTURE*	p-PHENYLENEDIAMINE		RECOVERY
		PRESENT	REPORTED	
		per cent	*per cent*	*per cent*
1	A	46.76	46.0	98.4
		46.76	45.6	97.5
2	A	46.76	45.6	97.5
		46.76	45.5	97.3
3	A	46.76	45.9	98.2
		46.76	46.2	98.8
4	A	46.76	46.4	99.2
		46.76	46.4	99.2
		46.76	46.8	100.1
4	B	33.0	32.9	99.9
		33.0	32.8	99.4
		33.0	32.7	99.1
5	B	33.0	33.0	100.0
		33.0	33.1	100.3
		33.0	32.9	99.7
6	B	33.0	32.4	98.2
		33.0	32.4	98.2
		33.0	32.6	98.8

* Composition of mixtures:

	A	B
	Per cent	*Per cent*
p-Phenylenediamine	46.76	33.0
Metol	27.99	33.0
Na_2SO_3 anhydrous	25.25	34.0

Because of the large molecular weight of the residue (2844.06 against that of *p*-phenylenediamine, 108.08) very small quantities of *p*-phenylenediamine can be determined.

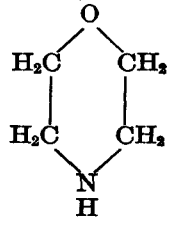
SUMMARY

A method is presented for the determination of *p*-phenylenediamine alone or in mixtures. The amine is precipitated with silicotungstic acid; and from the weight of the precipitate, incinerated at 500° C., the corresponding weight of the amine is calculated. Typical results are given.

DETERMINATION OF MORPHOLINE

By IRWIN S. SHUPE (Cosmetic Division,* U. S. Food and Drug Administration, Baltimore, Md.)

Morpholine, a heterocyclic secondary amine, has been proposed for use in cosmetics.[1] It has the following structural formula:



This amine is a colorless, slightly viscous liquid with an ammoniacal odor and alkaline properties. It boils at 128° C. and is soluble in water, alcohol, and ether. The free base combines with fatty acids to form soaps, which are efficient emulsifiers for such preparations as cosmetic creams. Because of its alkalinity and slow volatility it is adaptable for use in permanent wave solutions and has been patented for such use.[2]

Some of the chemical reactions of morpholine have been studied, and methods for its detection and determination are described.

EXPERIMENTAL

Preparation of pure morpholine and morpholine sulfate.—Redistilled morpholine base, and morpholine sulfate recrystallized from alcohol and

TABLE 1.—*Analytical data on morpholine and morpholine sulfate*

	NITROGEN		PURITY BY TITRATION	
	THEORY	FOUND		
	per cent	*per cent*	*per cent*	
Morpholine base (B.P. 127–8°C.)	16.08	16.1	99.8	
			H_2SO_4	
			THEORY	FOUND
			per cent	*per cent*
Morpholine sulfate	10.29	10.3	36.0	35.9

* D. Dahle in charge.

[1] Soap, Perfumery & Cosmetics, Buyers Guide & Cyclopaedia, United Trade Press Ltd., London (1939), p. 12.

[2] Wilson & Morin, Assignors to Carbide and Carbon Chemicals Corp., U. S. Pats. 2,154,924–5, April 18, 1939.

water, were used as reference standards. The sulfate was dried in a vacuum desiccator because heating at 100° C. caused a loss in weight with an increase in free sulfuric acid.

Table 1 gives analytical results showing the purity of the base and the sulfate thus prepared.

Titration and pH.—Preliminary tests with morpholine indicated that the base could be titrated with acids and methyl red indicator. A water solution of morpholine sulfate was neutral to methyl red. The *p*H of various concentrations of morpholine and its sulfate is shown in Table 2.

TABLE 2.—*The pH of morpholine solutions*

WATER SOLUTION OF—	*p*H
10.0% morpholine base	11.1
5.0% morpholine base	10.9
1.0% morpholine base	10.5
1.0% morpholine sulfate	4.90
0.5% morpholine sulfate	4.95

A weighed portion of morpholine was titrated with 0.1 *N* sulfuric acid. The *p*H at several points in the titration is shown in Table 3.

TABLE 3.—*Titration of morpholine (1.85 grams in 25 ml. of water)*

0.1 *N* H_2SO_4 ADDED	MORPHOLINE NEUTRALIZED	*p*H
ml.	*per cent*	
0	0	11.0
5.0	23.5	9.0
10.0	47.0	8.6
15.0	70.5	8.2
20.0	94.0	7.3
21.0	98.6	6.7
21.1	99.1	6.1
21.2	99.5	5.6
21.3	100.0	4.9
21.4		4.3
21.5		3.8
22.0		3.1
23.0		2.7
24.0		2.4

Figure 1, illustrating the critical part of the titration curve, and the analytical results show that methyl red is a satisfactory indicator for the titration of morpholine.

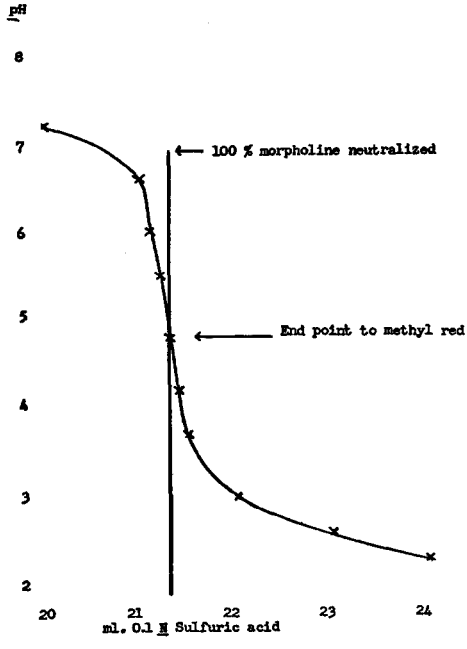


FIG. 1.—TITRATION OF MORPHOLINE

Steam distillation.—Acidified solutions of morpholine can be evaporated to small volumes without loss, but in alkaline solutions or in solutions of the base in water morpholine is volatile. It is stated[3] that a dilute solution of morpholine distils to contain substantially the same composition in the distillate as in the original solution. However, the rate of removal of morpholine by steam distillation may be greatly accelerated with excess alkali. Table 4 shows some relative rates of steam distillations.

With excess alkali morpholine could be quantitatively recovered in a small volume of distillate. When the distillate was well cooled, no acid was required as a retainer to prevent loss from volatilization (as in ammonia determinations). A drop of methyl red indicator was added to the distillate, which was titrated directly with standard sulfuric acid.

TABLE 4.—*Rates of steam distillation of morpholine*

EXP. NO.	SOLUTION CONTAINED—	VOLUME OF DISTILLATE	RECOVERY OF MORPHOLINE
		ml.	*per cent*
A	100 mg. of morpholine in 20 ml. of water (steam distilled)	10	15
		20	28
		40	43
		90	90
B	100 mg. of morpholine in 20 ml. of aqueous 25% w/v NaOH (steam distilled)	10	99.2
		20	99.6
C	100 mg. of morpholine in 150 ml. of water containing 25 ml. of 50% w/v NaOH (direct distillation in 500 ml. Kjeldahl flask)	120	97.4

[3] The Merck Index, Merck & Co., Inc., Rahway, N. J., 5th ed. (1940), p. 367.

Extraction.—Although morpholine in all proportions is miscible with water, it may be extracted with ether from solutions containing excess alkali. From an aqueous solution containing 20 per cent or more of sodium hydroxide morpholine can be extracted with ether without removal of any fixed alkali. Thus the usual requirement of washing an ether extract with water can be avoided, and a quantitative extraction of morpholine is possible.

The ether solution of morpholine may be mixed with water and titrated with standard acid or it may be converted to the dithiocarbamate by treatment with carbon disulfide.

Reactions of morpholine and tests for identity.—With silver nitrate morpholine reacts similarly to ammonia, forming a precipitate that dissolves in excess of the base. However, it differs from ammonia in its reactions

TABLE 5.—*Microchemical tests*

REAGENTS	DILUTION OF MORPHOLINE
Modified Kraut's*	to 1 in 800
Platinic chloride	1 in 25
Gold chloride	1 in 25
Silicotungstic acid	1 in 25
Phosphotungstic acid	1 in 1000
Reinecke salt†	1 in 300

* Dissolve 7 grams of bismuth subcarbonate in 20 ml. of conc. HCl. Add this mixture to a solution of 28 grams of KI in 50 ml. of water and dilute to 100 ml.
† Ammonium tetrathiocyano-diammono-chromate.

with copper, nickel, and cobalt salts, with which it forms no soluble complexes. Nessler's reagent does not react with morpholine, and therefore it may be used for the detection of ammonia in morpholine distillates.

A modified Simon's color test may be made with sodium nitroprusside and acetaldehyde reagent. This reagent is considered specific for secondary aliphatic amines.[4] With morpholine, a blue color is formed in dilutions up to 1 in 700. Ammonia gives a light orange color with the reagent and decreases the sensitivity of the test. Morpholine, in a dilution of 1 in 200, may be detected in the presence of ten times its weight of ammonium sulfate, however.

Crystalline precipitates, useful for microchemical tests, are formed in neutral or slightly acid solutions with the reagents shown in Table 5.

Kraut's reagent and Reinecke salt are especially useful in differentiating morpholine from ammonia.

Sulfonyl derivatives.—The benzene sulfonyl and the *p*-brombenzene sulfonyl derivatives were found to be stable and easily prepared. Detailed directions for their preparation are given later. These methods may be

[4] Feigl, Qualitative Analysis by Spot Tests, Nordemann Publishing Co., Inc., N. Y. 2nd English ed. (1939), p. 307.

used for the quantitative estimation of morpholine. The benezene sulfonyl derivative is slightly soluble in water and soluble in organic solvents, and it melts at 119° C. The *p*-brombenzenesulfonyl derivative is insoluble in water and soluble in organic solvents, and it melts at 153° C. Both derivatives can be dried at 100° C. Being derivatives of a secondary amine, they are stable in alkaline solutions. The sulfonamides from ammonia are soluble in alkali, and the methods are therefore applicable in the presence of ammonia.

Derivatives with carbon disulfide.—Morpholine reacts with carbon disulfide to form a crystalline dithiocarbamate from either an aqueous or an ether solution. The dithiocarbamate is practically insoluble in ether, slightly soluble in alcohol, and soluble in water. It sublimes without melting at temperatures above 100° C.

When an aqueous solution of the dithiocarbamate is treated with potassium ferricyanide, the ferricyanide is reduced and a water-insoluble derivative separates. This derivative, which is probably a thiuram disulfide, can be recrystallized from hot alcohol, and it melts at 150–1° C. The same derivative may be obtained direct from an aqueous solution of morpholine and ferricyanide by treatment with carbon disulfide. (This reaction also serves as a rather sensitive test for carbon disulfide. A solution of 1 part of carbon disulfide in 5,000 parts of water yields a crystalline turbidity with excess morpholine and potassium ferricyanide.)

METHODS

Qualitative Tests

Microchemical tests.—Place a drop of a water solution of morpholine sulfate of the concentration specified below on a glass slide. Add a drop of the reagent and examine the crystals formed with a microscope. Compare with a known specimen of morpholine.

CONCENTRATION OF MORPHOLINE SULFATE	REAGENT
1 in 200	Modified Kraut's
1 in 200	Reinecke salt (sat'd. water solution)
1 in 1000	Phosphotungstic acid (5% in water)

Color test.—To a solution containing about 0.2% morpholine or its salt, add an equal volume of a mixture containing 1 gram of sodium nitroprusside and 10 ml. of acetaldehyde in 100 ml. of water. Add sufficient 2% $NaHCO_3$ solution to assure an alkaline reaction. After a few seconds a blue color is formed, which gradually fades to a brown.

Quantitative Procedures

Steam distillation.—Transfer a measured portion of sample to a flask fitted for steam distillation. Add an equal volume of 50% w/v NaOH solution and steam distil. Collect about 25 ml. of distillate for quantities up to 100 mg. of morpholine. Test

additional distillate for complete evolution of morpholine. Add a drop of methyl red indicator to the distillate and titrate with standard H_2SO_4.

1 ml. of 0.1 *N* H_2SO_4 = 8.7 mg. of morpholine.

Benzene sulfonyl derivative.—In a glass-stoppered flask place an aliquot of the titrated steam distillate containing 10–50 mg. of morpholine. Evaporate to approximately 5 ml. if necessary. Add 0.5 ml. of benzene sulfonyl chloride and 10 ml. of 10% w/v NaOH. Shake intermittently for 30 minutes. Heat to boiling and shake to destroy excess benzene sulfonyl chloride. Cool, transfer to a separatory funnel, and extract with four 30 ml. portions of $CHCl_3$. Wash the $CHCl_3$ extracts with 10 ml. of water and filter through a pledget of cotton into a tared dish. Evaporate the $CHCl_3$ on a steam bath and dry in an oven at 100° C. for 30 minutes. Weigh the benzene sulfonyl morpholine. Make appropriate corrections for a blank on the reagents.

Benzene sulfonyl derivative × 0.383 = morpholine.

Recrystallized from alcohol and water, the derivative melts at 119° C.

p-Brombenzene sulfonyl derivative.—Make slightly acid an aliquot of the titrated steam distillate containing 5–25 mg. of morpholine and evaporate to about 10 ml. if necessary.

Dissolve approximately 0.25 gram of the *p*-brombenzene sulfonyl chloride in 10 ml. of acetone and add to the solution of morpholine. Then add approximately 2 grams of powdered $NaHCO_3$. Stir the mixture, and let stand for 15 minutes; heat to boiling on a steam bath and add 15 ml. of water. Continue the heating for about 15 minutes until the acetone has evaporated. Add 25 ml. of 2% w/v NaOH solution and let stand at about 4° C. for a few hours or overnight. Collect the precipitate in a tared Gooch crucible and wash with water. Dry in an oven at 100° C. for 1 hour and weigh the *p*-brombenzene sulfonyl morpholine.

p-brombenzene sulfonyl derivative × 0.285 = morpholine.

The derivative melts at 153° C.

Extraction and conversion to dithiocarbamate.—To a measured portion of a solution of morpholine or its salts, add an equal volume of 50% w/v NaOH. Extract with peroxide-free ethyl ether. If separatory funnels are used, extract with six 30 ml. portions of ether for a maximum of 30 ml. of aqueous alkaline solution. (A continuous extractor, equipped with an efficient reflux condenser, may be used.)

Filter the ether extracts through a pledget of cotton into a tared evaporating dish. (Do not wash the extracts with water.) Add 5 ml. of CS_2 to the ether extract, let stand 10 minutes, and evaporate on a steam bath. Permit the last portions of solvent to evaporate spontaneously. Dry in a desiccator and weigh the morpholine morpholyl dithiocarbamate.

The dithiocarbamate derivative × 0.696 = morpholine.

The derivative sublimes without melting.

Dissolve a portion of the dithiocarbamate in a small volume of water and add an excess of a 5% solution of $K_3Fe(CN)_6$. The crystalline precipitate formed, recrystallized from alcohol, melts at 150–1° C.

The methods for the sulfonyl derivatives were applied to solutions of morpholine of known concentration and to mixtures containing ammonia. Recovery data are shown in Tables 6 and 7. Recoveries by extraction and conversion to the dithiocarbamate are shown in Table 8.

Recovery of morpholine from creams and lotions.—The isolation of morpholine from creams and lotions is often complicated by the presence of other emulsifying agents. A preliminary separation for their removal may be necessary. For creams the samples may be heated with 10 ml. of 5 per cent hydrochloric acid to break emulsions and to decompose soaps. The

oily material may then be dissolved in ether and the aqueous extract subjected to steam distillation.

TABLE 6.—*Recovery of benzenesulfonyl derivative of morpholine*

EXP. NO.	CONTAINED—		WT. OF DERIVATIVE	RECOVERY OF MORPHOLINE
		mg.	*mg.*	*per cent*
1	Morpholine	10	24.4	93.0
2	Morpholine	20	49.9	96.0
3	Morpholine	30	76.0	97.0
4	Morpholine	40	101.9	97.7
5	Morpholine	50	130.0	99.6
6	Morpholine	10		
	$(NH_4)_2SO_4$	100	24.2	93.0
7	Morpholine	50		
	$(NH_4)_2SO_4$	10	130.2	99.6
8	$(NH_4)_2SO_4$	100	0	0

TABLE 7.—*Recovery of p-brombenzenesulfonyl derivative of morpholine*

EXP. NO.	CONTAINED—		WT. OF DERIVATIVE	RECOVERY OF MORPHOLINE
		mg.	*mg.*	*per cent*
1	Morpholine	5	16.5	94.0
2	Morpholine	10	34.1	97.0
3	Morpholine	15	51.0	96.7
4	Morpholine	20	65.9	98.5
5	Morpholine	25	87.6	99.8
6	Morpholine	5		
	$(NH_4)_2SO_4$	40	16.6	94.0
7	Morpholine	25		
	$(NH_4)_2SO_4$	10	87.7	100.0
8	$(NH_4)_2SO_4$	40	0	0

TABLE 8.—*Recovery of morpholine by extraction and conversion to dithiocarbamate*

EXP.* NO.	CONTAINED MORPHOLINE SULFATE	WT. OF DITHIOCARBAMATE	RECOVERY OF MORPHOLINE
	mg.	*mg.*	*per cent*
1	20	18.0	97.7
2	50	45.0	97.8
3	100	91.8	99.9
4	20	17.2	93.8
5	50	44.1	96.0
6	100	90.2	98.2

* Experiments 1–3: continuous extractor, 10 ml. aqueous layer.
Experiments 4–6: separatory funnels, 20 ml. aqueous layer.

Two types of creams and two of lotions were prepared to contain, in addition to morpholine, the other ingredients listed. Lotion A was acidified with hydrochloric acid and extracted with ether before a steam distillation

on the aqueous layer was made. Lotion B was steam distilled without preliminary treatment.

		Per cent
Vanishing cream base:	stearic acid	15
	cetyl alcohol	3
	glycerin	7
	water	75
Cold cream base:	beeswax	12
	paraffin	10
	mineral oil	44
	water	34
Lotion A:	borax	5
	sodium sulfite	5
	gum acacia	0.5
	glycerin	1
	oleic acid	0.5
	sulfonated oil	2
	mineral oil	1
	water	85
Lotion B:	Same as "A" except that it contained more water and oleic acid, sulfonated oil, or mineral oil.	

The recovery of morpholine from these mixtures is shown in Table 9.

TABLE 9.—*Recovery of morpholine by steam distillation from creams and lotions*

EXP. NO.	CONTAINED—	RECOVERY OF MORPHOLINE
		per cent
1	0.2% morpholine incorporated in a 5 gram sample of vanishing cream base	98
2	0.2% morpholine incorporated in a 5 gram sample of cold cream base	95
3	3% morpholine in 5 ml. of Lotion A	97
4	3% morpholine in 5 ml. of Lotion B	99

SUMMARY

Methods for the isolation and determination of morpholine, applicable to cosmetic creams and lotions, were studied.

These methods include steam distillation and titration with acid, the quantitative conversion to the benzenesulfonyl and *p*-brombenzenesulfonyl derivatives, and the extraction and conversion to the dithiocarbamate. A color test and microchemical tests for identity are also described. Typical results are reported.

BOOK REVIEWS

General College Chemistry. By LEON B. RICHARDSON and ANDREW J. SCARLETT. Henry Holt and Company, New York. 1940. 683 pp. $3.75.

This book is the second revision (third writing) of Richardson's General Chemistry, a text that is well-known in its field. According to the preface "the most radical change in this revision is the discussion of modern views of the nature of acids, bases and salts and of the mechanism of the process of ionization. These developments are not only set forth in detail, but they are used consistently in the discussion of practical topics which follows." The authors are to be congratulated on discarding the outmoded views on these subjects and using from the beginning and consistently the Brønsted system and other modern concepts. There is no excuse for perpetuating the confusion that most of us have had to contend with in reorienting ourselves after the habit-forming use of the older ideas for a long time.

In bringing the subject matter up to date, the authors have also succeeded well in retaining the object of the original text: "The student should gain from his study of inorganic chemistry in college an acquaintance with the way in which the scientist thinks; with the theoretical principles which bind the science together and reveal the relationship between matters seemingly far apart. Secondly, he should acquire a knowledge of what the chemist has done and is doing for the comfort and convenience of mankind."

The reviewer wishes that the authors had not left the impression that they regard the dilution and artificial coloring of glacial acetic acid for sale as vinegar as one of these contributions by the anemic statement that this is "a practice not considered to be legitimate" (p. 632).

The binding and typographical set up are excellent. This book is undoubtedly among the best of its type.—EDWARD O. HAENNI.

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