The results indicate that those portions of each series kept in a dark closet had undergone no material change. Series I apparently had not suffered any change when exposed to ultraviolet light (*b*) or sunshine (*c*) if judged by the amount of ether residue. However, this was not the case because the color of the series was progressively darker from (*a*) to (*b*) to (*c*). The color of the samples (*a*), (*b*) and (*c*) was not appreciably changed by the extraction with ether. This indicated that, although a change had taken place, the dark product of photochemical action was not extractable with ether. On the other hand, the color of samples (*b*) and (*c*) of Series III was lightened by the ether extraction because of the fact that a large proportion of the extract was the added stearic acid which was likewise discolored by the exposures. However, the color of these samples after the removal of the ether soluble matter was markedly darker than the samples in Series I and II similarly exposed. It is apparent from this experiment that stearic acid increases the amount of ether insoluble as well as the ether soluble pigment.

The larger ether residues obtained in the case of Series II when exposed to ultraviolet light and to sunshine indicated the possibility that the alcohol-acetone treatment of sulfanilamide caused the formation of some complex which is readily affected by ultraviolet or solar radiation. This was shown not only by the higher residues but also by an appreciably greater discoloration than those produced in Series I.

An attempt to determine the character of the residues by the colorimetric procedure of Rosenthal and Bauer (1) failed to give positive results for the usual oxidation products of sulfanilamide; namely, hydroxylamine, nitroso or nitro compounds. A coupling test with sulfanilic acid, however, indicated the presence of small amounts of phenolic bodies—probably para-aminophenol. Also, this test indicated appreciable amounts of phenolic bodies in the residue obtained by the ether extraction of the sulfanilamide powder used in this series of tests (Brand B). A comparison of this residue with the original drug by Marshall's method indicated that the residue was approximately 85 per cent sulfanilamide. The remaining 15 per cent was impurity and calculated to 0.038 per cent of the original sample. This latter figure is comparable to the 0.04 per cent impurity found in the various portions of Series I, II and III (see Table IV).

CONCLUSIONS

1. Investigation of sulfanilamide tablets on the market failed to reveal decomposition to any appreciable extent.

2. The small quantities of impurities found are traceable, for the most part, either to the residual impurities in the brand of drug, or to added "lubricant" used in the process of tablet manufacture.

3. Under extreme conditions of exposure to ultraviolet light or to sunshine, sulfanilamide may undergo appreciable photochemical change, especially in the presence of impurities. The use of stearic acid as a "lubricant" in sulfanilamide tablets is not advisable as it apparently promotes photochemical decomposition of the drug.

Grateful acknowledgment is made of the assistance of Mr. Edward M. Lutz in preparing the photomicrographs.

REFERENCE

(1) Rosenthal, S. M., and Bauer, H., *Public Health Reports*, 54 (1939), 1880.

A Study of the Assay of Blaud's Pills and Effects of Various Sugars upon Their Stability*

By M. L. Neuroth† and C. O. Lee‡

This study was undertaken for the purpose of examining the effects of various sugars upon the ferrous iron in Blaud's Pills. The accuracy of several methods of assay for this preparation have also been investigated.

The assay for Blaud's Pills in the U. S. Pharmacopœia XI permits the use of dichromate with diphenylamine T.S. as the indicator. The Second Supplement permits the use of ceric sulfate with *ortho*-phenanthroline T.S. as the indicator.

Recent investigators have shown that ceric sulfate is a satisfactory oxidizing agent for ferrous iron determinations. Several indicators have given good results with it.

EXPERIMENTAL

THE PREPARATION OF THE PILL MASSES

The official formula for Pills of Ferrous Carbonate is as follows:

Ferrous Sulfate, in clear crystals	16 Gm.
Potassium Carbonate	8 Gm.
Sucrose, finely powdered	4 Gm.

* An abstract of a thesis presented to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Master of Science.

† Instructor in Pharmacy, Ohio Northern University.

‡ Professor of Pharmacy, Purdue University.

Tragacanth, fine powdered	1 Gm.
Althea, in very fine powder	1 Gm.
Glycerin, a sufficient quantity	
Distilled, water, a sufficient quantity	
To make	100 pills

The formula was made into a mass according to the official directions. This formula and procedure were used for preparing six other masses substituting as many different sugars for sucrose. The sugars which were selected for study may be classified as follows:

I. Monosaccharides
  1. Pentoses
    (*a*) Arabinose
    (*b*) Rhamnose
  2. Hexoses
    (*a*) Galactose
    (*b*) Fructose

II. Disaccharides
  (*a*) Sucrose
  (*b*) Maltose

III. Trisaccharide
  (*a*) Raffinose

Each of the seven masses was divided into eight portions. One portion of each was labeled as the control. A second portion of each was assayed immediately. The remaining 42 portions were placed separately in screw-capped vials. These were assayed at intervals of from two to six weeks through the year, making seven series of assays in all.

VOLUMETRIC SOLUTIONS AND INDICATORS

Furman (1), Willard and Young (2), Ferrey (3), Watt (4), and Lyons and Appleyard (5) all studied the possibilities of ceric sulfate standard solution for use in the estimation of ferrous iron. They concluded that it was a good reagent for the titration of ferrous iron in the presence of organic reducing substances. For this reason we have chosen to use it in this study.

A. Solutions.—1. Ceric ammonium sulfate approximately 0.1*M* (mol. wt. 628) $Ce(SO_4)_2.2(NH_2SO_4.2H_2O)$ in 1*N* H_2SO_4.

Dissolve 63 Gm. of the hydrated salt in about 300–500 ml. of water containing 28 ml. of concentrated sulfuric acid. When solution is complete, dilute to one liter. Standardize the solution against 99.8 per cent arsenic trioxide in the following manner: Place 0.15–0.22 Gm. of arsenic trioxide, previously dried to constant weight at 100° C. and accurately weighed, in a 250-ml. Erlenmeyer flask. Add 15 ml. of 2*N* sodium hydroxide and warm the mixture gently to hasten solution. When the arsenic trioxide has completely dissolved, cool it to room temperature and add 25 ml. of sulfuric acid (1:5). Dilute the solution to 100 ml. and add 3 drops of 0.01*M* osmium tetroxide (0.25 Gm. OsO_4 in 100 ml. of 0.1*N* sulfuric acid) as a catalyzer. A blank should be run and a correction made for the amount of the osmium tetroxide. Titrate the solution with the ceric ammonium sulfate using one drop of *ortho*-phenanthroline ferrous ion solution as an internal indicator. Each ml. of tenth normal ceric ammonium sulfate solution is equivalent to 0.004946 Gm. of arsenic trioxide (4, 6).

The ferrous iron content was also determined by titration with potassium dichromate using diphenylamine as an internal indicator.

2. An approximately 0.1*N* solution of potassium dichromate was prepared by direct weighing and the normality checked by titration against a dissolved sample of pure iron wire using potassium ferricyanide as an external indicator.

B. Indicators.—Three internal indicators were selected for comparison as to accuracy and color change at the end-point when ceric ammonium sulfate was used. Each indicator solution was used in turn. They are as follows:

1. *Ortho*-phenanthroline ferrous complex ion (0.025*M*). This indicator solution was purchased ready for use.

2. Xylene Cyanole F.F. The strength of this indicator was 0.1 per cent in water, one-half ml. being added in each titration near the end-point. The color change should be from a light green to a pink.

3. Phenylanthranilic Acid. Dissolve 0.11 Gm. of phenylanthranilic acid and 0.3 Gm. of anhydrous sodium carbonate in 100 ml. of distilled water. The solution is colorless when freshly prepared, but changes to a pink after several months. The slight oxidation does not affect the indicator action of the solution. Two or three drops are used for each titration. The color change at the end-point is from colorless to a deep pink when an excess of ceric ammonium sulfate is present.

4. Diphenylamine T.S. Dissolve 1 Gm. of the substance in 100 ml. of reagent sulfuric acid. This solution was used as an internal indicator with the dichromate solution for the estimation of the ferrous ion in the pill masses.

In order to obtain a check on the various methods of assay employed for the determination of the ferrous iron content of the pill masses, additional analyses of the ferric and total iron were made.

C. Preparation and Standardization of Stannous Chloride Solution.—Thirty-five grams of stannous chloride were added to 300 ml. of concentrated hydrochloric acid and the volume made up to 2000 ml. The solution was filtered into an aspirator bottle arranged in such a way that an atmosphere of carbon dioxide could be maintained throughout the entire procedure.

To determine the strength of the solution place 0.2 Gm. of pure iron wire, cleaned, dried and accurately weighed in 25 ml. of 25 per cent sulfuric acid. Add 1 Gm. of potassium chlorate and boil gently until solution is complete and all the iron is oxidized to the ferric condition. Continue the heat until the excess chlorate has been decomposed. It is necessary that there be no free chlorine present. Cool

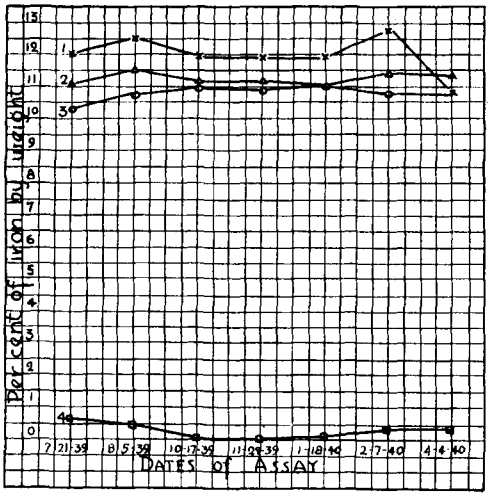

Fig. 1.—Pills of Ferrous Carbonate (Arabinose).

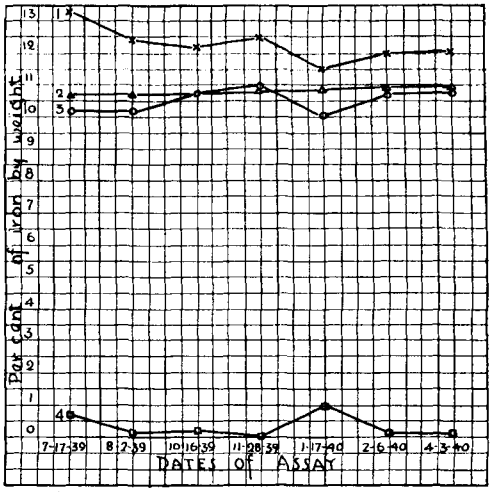

Fig. 2.—Pills of Ferrous Carbonate (Fructose).

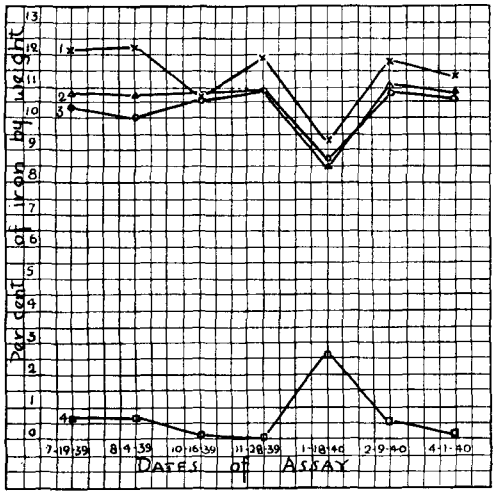

Fig. 3.—Pills of Ferrous Carbonate (Galactose).

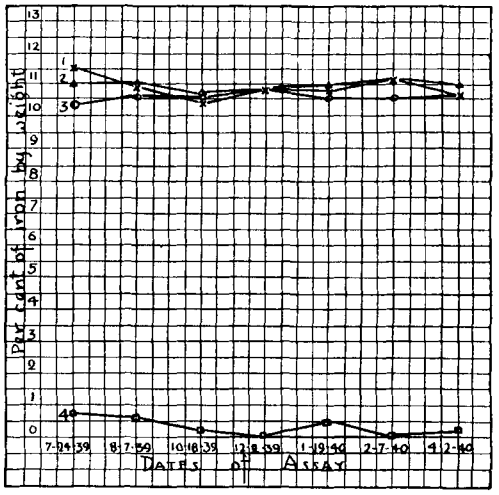

Fig. 4.—Pills of Ferrous Carbonate (Maltose).

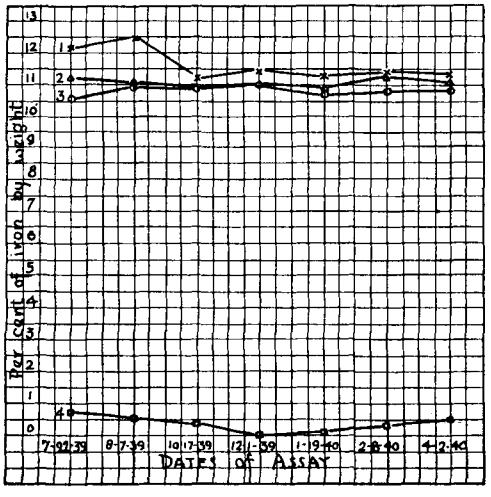

Fig. 5.—Pills of Ferrous Carbonate (Raffinose).

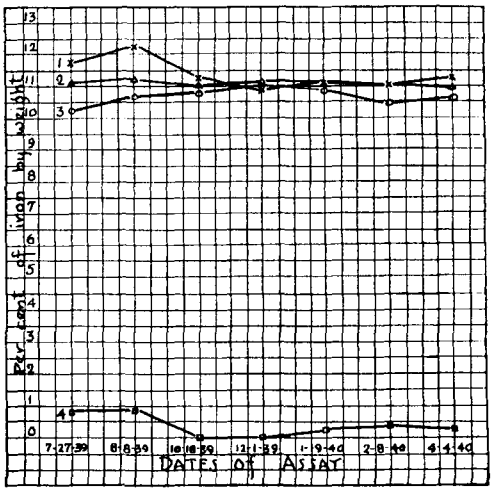

Fig. 6.—Pills of Ferrous Carbonate (Rhamnose).

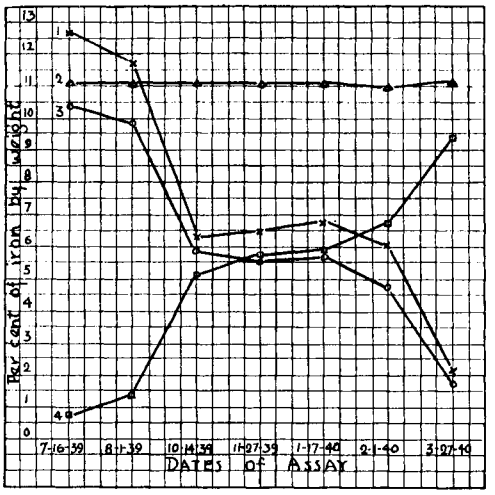

Fig. 7.—Pills of Ferrous Carbonate, U. S. P. XI (Sucrose).

the solution and dilute to approximately 75 ml. Heat again to redissolve the residue which has formed. Titrate the hot solution immediately in an atmosphere of carbon dioxide with the stannous chloride solution until the color of the ferric ion disappears, then add 2 ml. in excess. Back titrate the excess tin solution with an iodine solution previously standardized against the stannous chloride solution. Determine the stannous chloride-iodine solution ratio by titrating 2 ml. of the tin solution with the iodine solution using a freshly prepared starch T.S. as the indicator.

D. Analysis of the Pill Masses.—In order to avoid errors due to possible variations of small quantities the analysis was run on the pill mass rather than on a few pills made from the mass. Errors due to lack of uniformity of pills is thus minimized. The following procedure was applied. A portion of the pill mass, approximately 4 Gm., was dissolved in 37.5 ml. of cold 25 per cent *W/V* sulfuric acid and diluted to 250 ml. and the following titrations made:

1. Ferrous Iron: (*a*) Titrate 25 ml. of the solution against the standardized ceric ammonium sulfate solution using ferrous *ortho*-phenanthroline as an internal indicator.

(*b*) Titrate 25 ml. of the solution against the ceric ammonium sulfate solution using xylene cyanole F.F. as the indicator.

(*c*) Titrate another 25 ml. sample against the standardized potassium dichromate solution using diphenylamine T.S. as an internal indicator.

(*d*) Titrate another 25 ml. sample against the ceric ammonium sulfate solution using phenylanthronilic acid as the indicator.

2. Ferric Iron. (*a*) Heat 25 ml. of the solution and titrate against the standardized solution of stannous chloride in an atmosphere of carbon dioxide. Add enough of the tin solution to dissipate the color of the ferric ion, then add just a slight excess. Again warm the solution to insure complete reduction of the iron. Titrate the excess tin against an iodine solution using a fresh starch T.S. (1 ml.) as an indicator.

3. Total Iron. (*a*) Add 1 Gm. of potassium chlorate to 25 ml. of the solution and heat strongly to oxidize all of the organic material present and to convert all of the iron to the ferric condition. Boil the solution vigorously to destroy the excess potassium chlorate and to remove any chlorine which might prevent the subsequent reduction of the iron. Dilute the solution to approximately 75 ml. and heat until the residue which has formed is dissolved. Adjust the solution to a temperature of 75° and titrate against the standardized solution of stannous chloride in an atmosphere of carbon dioxide. Add enough stannous chloride to dissipate the color of the ferric ion, then just a little excess. Warm the solution again to insure complete reduction of the ferric ion. Titrate the excess tin solution against an iodine solution using a fresh starch T.S. as an indicator.

Each titration throughout the entire series of assays was run in duplicate.

In regard to the advisability of starch T.S. as an indicator, Kolthoff and Furman (7) state that the sensitivity is 1×10^{-4} to 2×10^{-4} in iodide. The delicacy decreases with a rise in temperature. Starch serves best as an indicator at temperatures between 15° C. and 87° C. and it is oxidized by iodine at high temperatures. Organic substances diminish the sensitivity of the iodine-starch reaction. Every effort was made in this work to control all the factors which might affect the reactions at the end-point of the titration.

Sutton (6) advises a slight excess of stannous chloride solution and back titration with standard iodine solution.

TITRATION DATA

The results of 588 titrations are represented in the seven figures which follow.

Graph No. 1 of each figure represents the values obtained for ferrous iron determined by titrating with potassium dichromate solution using diphenylamine as the indicator.

Graph No. 2 of each figure records the total per cent of iron measured by oxidizing the mass and subsequently titrating with stannous chloride solution to an excess, and back-titrating with iodine solution and starch T.S. as the indicator.

Graph No. 3 of each figure really represents *three* titrations. Ceric ammonium sulfate solution was used as the oxidizing agent. In one case *ortho*-phenanthroline-ferrous complex was used as the indicator; phenylanthranilic acid and xylene cyanole F.F. were the other two indicators employed. Inasmuch as the values obtained in each case were practically the same, the average of the three determinations was recorded in the one graph.

Graph No. 4 of each figure shows the quantity of ferric iron found in the mass by using the stannous

chloride-iodine titration method without preliminary oxidation.

The iron content is expressed in percentage by weight based upon the original weight of each portion of the mass. The dates of each series of assays are indicated.

SUMMARY AND CONCLUSIONS

1. All of the masses except the one made with sucrose retained good color and plasticity throughout the several months of study. The freshly prepared sucrose mass had a fine appearance. By the end of the second week it had developed a reddish brown color. After nine months the mass was hard and much oxidized at the surface. Internally the color was grayish green with brown spots throughout. The assays indicated that the ferrous iron had almost completely oxidized to the ferric iron.

2. The masses made with maltose and rhamnose showed some crystallization at the surface after several months.

3. Lucas and Stevens (1903) and Greenish (1904) observed that reducing sugars were capable of changing the ferric iron in Blaud's Pills to the ferrous state. Our observations seem to bear out this fact.

4. The variations between the ferrous and ferric iron content are consistent in most cases, as is indicated by the results for total iron except for galactose.

5. There is need for further investigation into the role played by reducing sugars in preventing the oxidation of ferrous iron in Blaud's Pills.

6. The ceric ammonium sulfate assay is, without doubt, more accurate than the dichromate procedure.

7. There seems to be no difference between *ortho*-phenanthroline-ferrous complex ion and phenylanthranilic acid as internal indicators for use with ceric ammonium sulfate.

8. Xylene cyanole F.F. was not wholly reliable as an internal indicator when used with ceric ammonium sulfate. In some cases the color change at the end-point failed to develop. This gave erratic results.

9. Potassium dichromate with diphenylamine T.S. as an internal indicator invariably gave high results on the estimation of ferrous iron.

REFERENCES

(1) Furman, N. H., *J. Am. Chem. Soc.*, 50 (1928), 755.

(2) Willard, H. H., and Young, P., *Ibid.*, 50 (1928), 1322.

(3) Ferrey, G. J. W., *Quart. J. Pharm. Pharmacol.*, 10 (1937), 351.

(4) Watt, L. A., *National Formulary Bull.* (Jan. 5, 1937), 2506.

(5) Lyons, C. G., and Appleyard, F. N., *Quart. J. Pharm. Pharmacol.*, 10 (1937), 343.

(6) Sutton, F., "Volumetric Analysis" (1935).

(7) Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis," Vol. II (1929).

(8) Lucas, E. W., and Stevens, H. B., *Pharm. J.*, 71 (1903), 400.

(9) Greenish, H. G., *Ibid.*, 73 (1904), 734.

Phytosterol from the Buds and Fruit of the Tung Tree (*Aleurites fordii Hemsl.*)*

By Harold M. Sell and Albert H. Best

Work has been in progress at the U. S. Field Laboratory for Tung Investigations at Gainesville, Florida, on the physiological functions and nutrition of the tung tree at various periods of the year when major changes are taking place in the different tissues. In the course of this study, the waxlike substance found in the terminal bud was of interest. The literature (1, 2) does not report any work on the biochemical composition of the dormant tung bud. In this investigation a sterol has been isolated in a crystalline state from the bud and mature fruit and identified as phytosterol.

EXPERIMENTAL

Preparation of the Extract from Tung Buds.—In February of 1940, 7.1 lb. of buds[1] were collected from trees in the Alachua Tung Oil Orchards. The buds were cut into small pieces and extracted with petroleum ether for 24 hours. They were then ground to pass a 2-mm. mesh sieve and extracted for an additional 24 hours. Of this extracted material, 400 Gm. were saponified by refluxing with 350 ml. of 15% potassium hydroxide in 85% ethanol for 2 hours. After saponification the solution was diluted with 1200 ml. of water and extracted in a continuous

* Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Gainesville, Florida.

[1] We are indebted to Mr. Rolf Buckley of the Alachua Tung Oil Company for this material.