acteristic physical properties are described. The property of forming these bromosalts is correlated with the type of nitrogen in the base; some effects of steric factors and of substituents on the reactivity of the nitrogen of the alkaloid is noted in some cases, and it is shown that some prediction of the type of nitrogen in the base is possible from a study of these reactions.

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The Decomposition of Sulfanilamide in Tablets

*By Felice A. Rotondaro**

Through the courtesy of Dr. Paul Nicholas Leech of the American Medical Association, our attention was directed to a report of untoward reactions in four patients who had received sulfanilamide tablets showing a brownish discoloration. It was stated that substitution of another brand of sulfanilamide tablets eliminated the difficulty. In view of this report and the importance of sulfanilamide in the field of chemotherapy, investigation of the extent of the decomposition of sulfanilamide on the market and the factors concerned in such decomposition was undertaken.

A preliminary examination of the questionable tablets by extraction with acetone, followed by several recrystallizations, yielded a residue which was appreciably darker in color and which had a lower melting point than the residue similarly obtained from the second brand of tablets used. However, the chemical nature of the residues was not determined and no evidence was at hand to indicate their therapeutic significance.

It was decided that a survey of a fairly representative number of sulfanilamide tablets, as well as various brands of U. S. P. quality sulfanilamide powder on the market, would give some clue to the nature and significance of the residues. Nineteen samples representing thirteen brands of tablets, and seven samples representing four brands of U. S. P. quality powder were examined.

EXPERIMENTAL

The quantitative results obtained for sulfanilamide on all samples by the U. S. P. method showed that all were well within the limits of good manufacturing practice. However, this method of analysis is based on a general type reaction and is not specific for sulfanilamide. This is also true of methods based on the determination of an element such as nitrogen or sulfur. Therefore, small quantities of decomposition products or other impurities cannot be readily detected in the presence of large amounts of comparatively pure drug by the above methods.

Extraction of the tablets with anhydrous acetone followed by several recrystallizations, served to concentrate the impurity. The residue obtained from the acetone extractions and recrystallizations was shown to differ from similar residues from pure sulfanilamide and from tablets of sulfanilamide of known purity. The presence of an impurity was shown from the fact that the residue was of a dark brown color—in contrast to the pure white color of the pure drug—and that the melting point range was between 156–170° C. (Melting point of sulfanilamide = 164.5–166.5° C. U. S. P. XI.) Further, an examination of the solidified melt by polarized light showed that the crystal structure was broken up by a foreign substance; whereas, pure sulfanilamide yielded a beautiful continuous fan-like crystal branching from the point of crystallization.

From the fact that some crystals of pure sulfanilamide developed a brown color when exposed to rather bright daylight, it was inferred that the brown color of the tablets in question was due to some photochemical decomposition product of sulfanilamide.

A number of other brands of sulfanilamide tablets, about which no complaint was reported following their use, yielded acetone residues essentially similar to those obtained from the tablets reported to have caused untoward effects. This suggested that some substance commonly used by a number of manufacturers was the interfering agent in the determination of the melting point and optical characteristics of the acetone residues. It thus became apparent that

* Food and Drug Administration, Philadelphia, Pennsylvania.

fruitful results would depend upon a better separation, quantitative, if possible, of the tablet constituents, such as the "fillers" and "lubricants" as well as the isolation of the impurity from the sulfanilamide.

To eliminate as much of the sulfanilamide as possible from the small quantity of impurity, it was decided to use anhydrous ether as the solubility of sulfanilamide in this solvent is about 1 part in 1500 at about 30° C. Preliminary trials on tablets with this solvent yielded a much darker residue having a wider range of melting point than those obtained by the use of acetone. The method finally adopted for the systematic examination of all of the samples was as follows:

A representative number of tablets were ground to a relatively fine powder. Five gram portions of the powder were then placed in a 30-cc. sintered glass crucible and treated with 5 cc. of anhydrous ether; the wet sample was then thoroughly stirred with a glass rod for several minutes. The solvent was then drawn through by slight suction into a tared evaporating dish. Four more 5 cc. portions of ether were used as above. The combined ether extractions were then evaporated in a moderate current of air by placing the dish on the front ledge of the steam bath and lowering the door of the hood to about three inches above the dish. Finally, the last traces of the solvent were evaporated by placing the dish on top of the steam bath where the temperature did not exceed 70–80°. After cooling, the dish was weighed. The dish was again placed on the steam bath for a few minutes, then again weighed after cooling. If the weight did not differ by more than one half milligram, it was considered constant weight.

A portion of the residue was then placed between two cover slips and the melting range observed on a micromelting point apparatus. After complete fusion was obtained, the apparatus was allowed to cool gradually and the solidification point of the melt was observed. The crystallized residue was then examined by means of polarized light.

The results obtained by the above method are given in Tables I and II. The amount of residue varied from 0.24% to 0.43% for pure (?) sulfanilamide, and from 0.30% to 1.24% for the tablet samples.

The melting range of the ether residues from the U. S. P. quality sulfanilamide samples, with one exception, was fairly close to theory for the pure drug. This indicated that the residue consisted, for the most part, of sulfanilamide, and that only a small proportion was actually an impurity. No appreciable difference was found in the melting point of drug before and after the ether treatment. In contrast, the melting range of the ether residues from the tablet samples varied greatly.

The microscopic examination of the residues during the heating for the melting point determination showed the following sequence of changes:

(*A*) With untreated U. S. P. Sulfanilamide, Samples Nos. 20–26, inc. (see Figs. 1, 2 and 3):

1. The powder remained white until it began to melt, the melting point being quite sharp (164–166° C.).

2. On cooling to 140–145° C., the melt solidified in characteristic fan-like crystals with fracture lines perpendicular to the axis of extinction.

3. The crystalline solid appeared continuous and had no appreciable amounts of inclusions, extraneous matter or other irregularities.

(*B*) With ether residues from U. S. P. Sulfanilamide, Samples Nos. 20–26, inc., and tablets, Samples Nos. 5, 6, 13–19, inc. (see Fig. 4):

1. Slight sublimation at about 125° C. followed by slight yellowing of the residue.

2. Appreciable softening of some of the grains of the residue with further darkening at about 145°. The progressive softening of the residue was accompanied by slight decomposition as indicated by the evolution of gas.

3. Complete fusion resulted at about 155–160° C. The cooled (cir. 140° C.) melt showed the presence of a few dark bodies as inclusions in the otherwise characteristic, though slightly yellowish, crystalline mass.

(*C*) With the residues from the remaining samples of tablets, Samples Nos. 1–4, 7–12, inc. (see Figs. 5, 6, 7 and 8):

1. A few residues showed appreciable decomposition—as indicated by gas evolution and yellowing of the softening mass at as low a temperature as 70° C.

2. At about 100°, a portion of the melt ran off to the edge of the cover-slips. This melted portion was relatively colorless, while the portion left near the center of the cover-slips browned as it softened at about 145° C. At about 155°, numerous gas bubbles formed, indicating further decomposition. Complete fusion usually took place at about 160° C.

3. Upon cooling (cir. 130–140° C.), the browned melt in the center solidified to a fan-like crystalline mass very similar to residues in Series B. The condensation of the gas bubbles, however, left a number of small round holes in the mass. Also, a number of inclusion bodies—impurites and carbonized masses—were evident in some of the samples. The portion of the melt which ran off to the edge of the cover-slips solidified at much lower temperatures into feathery white crystals.

From the behavior of this last group of residues on melting, it became apparent that these extracts were mixtures of essentially two substances with rather wide differences in melting points. A nearly quantitative separation of the two fractions was accomplished by the following method:

A glass tube of 5–6 mm. diameter was drawn to a capillary and then a pledget of glass wool was placed in the shoulder between the capillary and the original size tubing. About 10 mg. of the low melting residue was then placed in the larger end of the tube and two 0.2 cc. portions of ether were slowly drawn through the tube. The ether extract was allowed to evaporate on a watch glass and weighed. This amounted to 60–70% of the original residue. The melting point of this extract proved to be quite

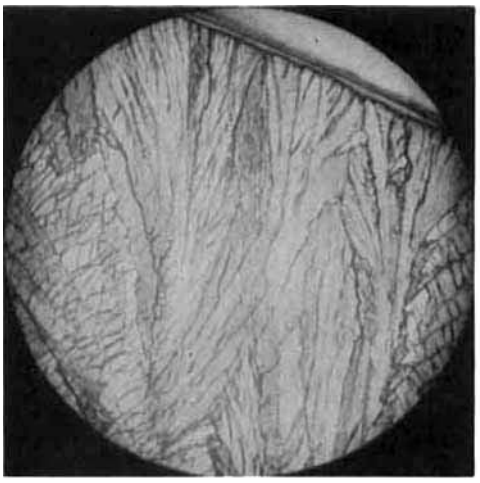

Fig. 1.—Sulfanilamide, U. S. P. Powder.

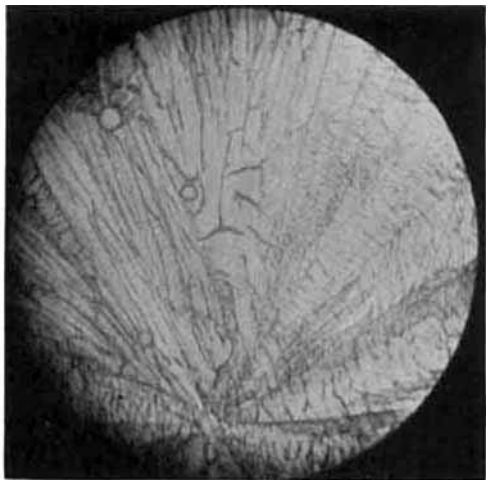

Fig. 2.—Sulfanilamide, U. S. P. Powder.

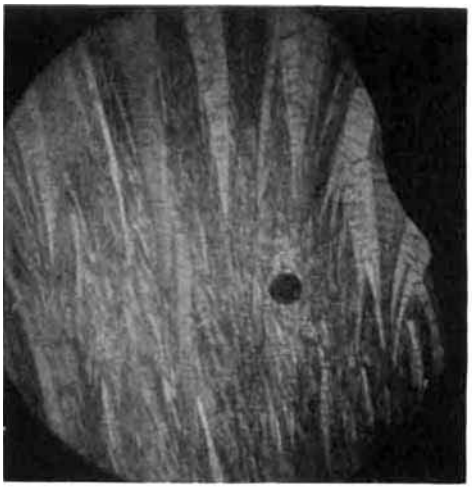

Fig. 3.—Sulfanilamide, U. S. P. Powder (Polarized Light).

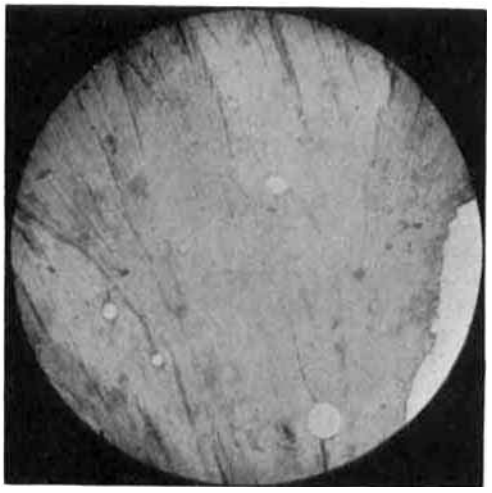

Fig. 4.—Ether Residue from Sulfanilamide, U. S. P. Powder.

Fig. 5.—Sulfanilamide Plus 0.5% Stearic Acid—Stored in Dark Closet.

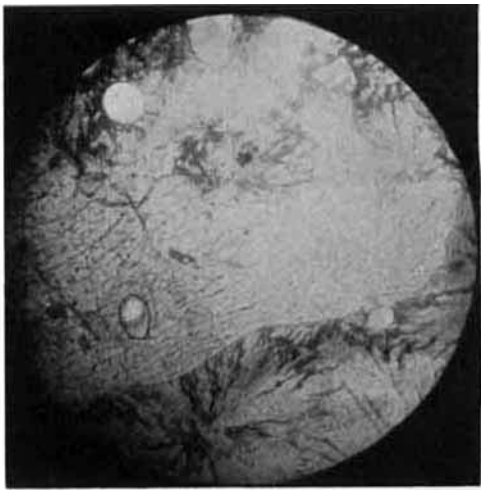

Fig. 6.—Sulfanilamide Plus 0.5% Stearic Acid—After Exposure to Sunlight for 25 hours.

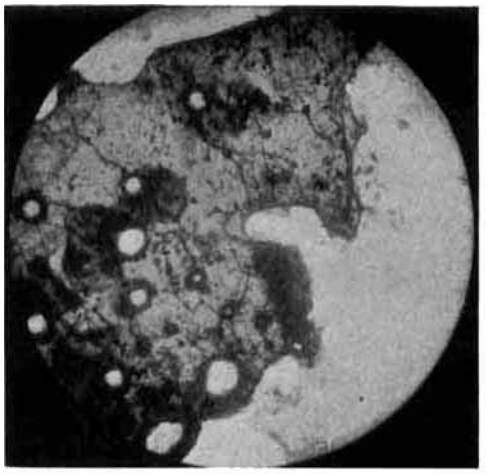

Fig. 7.—Ether Extract from Sulfanilamide Plus 0.5% Stearic Acid—After Exposure for 25 Hours to Ultraviolet Light.

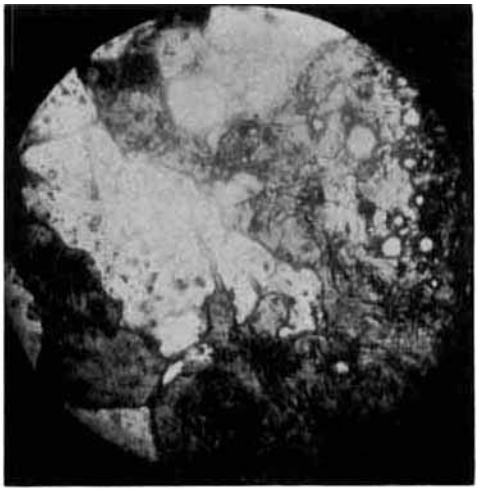

Fig. 8.—Ether Extract from Sulfanilamide Plus 0.5% Stearic Acid—After Exposure to Sunlight for 25 Hours.

sharp at 54–55° C. and its crystalline structure indicated stearic acid. (The use of stearic acid as a "lubricant" in these tablets was later confirmed by their manufacturer.) The residue left in the tube was next extracted with two 0.2 cc. portions of anhydrous acetone. The solvent evaporated and the residue recovered amount to 30–40% of the original residue. The melting point of this second fraction was quite sharp at 158–160° C. On cooling, the melt crystallized into the characteristic fan-like structure of sulfanilamide. Treatments of small portions of this residue with benzaldehyde and phenylhydrazine gave smooth rhombic plates similar to those obtained with pure sulfanilamide under like conditions.

Examination of the tablet samples for "fillers," by first removing the sulfanilamide and other soluble matter with anhydrous acetone, showed the presence of cornstarch and talc in most of the samples. A few samples showed the presence of either arrowroot or potato starch, and some contained lactose.

Table I summarizes the results from the tablet samples.

Table I.—Sulfanilamide Tablets

Sample Brand	No.	Assay, Gr./Tab.	Per Cent Label	Ether, Per Cent	Residue M. p., ° C.	Acid Stearic
A	1	7.23	96.8	0.89	90–150	+
	2	7.49	99.7	0.85	90–150	+
	3	4.97	99.4	0.88	90–150	+
	4	5.04	100.9	0.85	90–150	+
B	5	4.95	99.0	0.36	158–160	—
C	6	5.15	103.0	0.30	135–155	—
D	7	4.98	99.7	1.12	70–155	+
E	8	4.90	98.0	0.88	125–150	+
F	9	4.97	99.4	0.80	75–150	+
G	10	....	.....	1.20	65–155	+
	11	....	.....	1.24	65–155	+
H	12	....	.....	1.12	75–157	+
I	13	7.88	105.0	0.64	145–155	—
J	14	5.20	104.0	0.40	135–155	—
K	15	5.03	100.8	0.34	145–155	—

Table I.—*Continued*

L	16	....	.....	0.60	145–160	—
	17	....	.....	0.56	150–163	—
	18	....	.....	0.36	155–164	—
M	19	....	.....	0.45	157–162	—

The essential difference between tablet Sample No. 1, reported to have caused undesirable reactions, with Sample No. 5, which was of the same brand as used subsequently with good results, was their content of stearic acid. It is scarcely conceivable that this component as such could have been responsible for the ill effects noted since no similar effects have been reported from the use of a large number of other brands of sulfanilamide tablets containing stearic acid.

The results obtained with the U. S. P. quality sulfanilamide are summarized in the following table:

Table II.—U. S. P. Sulfanilamide Powder

Sample		U. S. P. Powder		Ether Residue	
Brand	No.	U. S. P. Assay	Melting Point	Melting Point	Per Cent
A	20	101.8	164–165	157–160	0.36
	21	100.7	164–165	160–163	0.26
B	22	100.9	164–166	163–165	0.26
	23	100.4	164–166	160–166	0.24
C	24	101.2	164–165	130–155	0.43
	25	100.8	165–167	158–163	0.36
D	26	101.5	164–166	163–165	0.24

The characteristics of the residues extracted by anhydrous ether from U. S. P. quality sulfanilamide indicated that the drugs contained some impurities before they were compressed into tablets. These impurities may be residues of the original ingredients used in the manufacture of the drug, or they may represent oxidation products of sulfanilamide, probably the result of photochemical action.

The effect of sunlight and of ultraviolet light on sulfanilamide was indicated by the following preliminary experiments:

I*a*. A portion of Brand A, U. S. P. quality drug was placed in an ordinary clear-glass screw-cap bottle and exposed to the light of a mercury vapor lamp for 18 hours.

I*b*. A similar sample was exposed out-of-doors for two weeks. The weather, for the most part, was cloudy and cool. The sample was then exposed to direct sunshine for 24 hours in an open Petri dish.

To another portion of the same brand of sulfanilamide as used above, 0.5 per cent of stearic acid was added and exposed.

II*a*. Similar to I*a*.—namely, 18 hours ultraviolet.

II*b*. Similar to I*b*.—namely, two weeks to daylight in a bottle, and 24 hours to direct sunshine in open Petri dishes.

A summary of the results of these experiments are presented in Table III.

In order to more effectively test the photochemical action of the direct rays of ultraviolet light and of sunshine, the following series of samples were prepared from a sample of Brand B sulfanilamide.

I. Brand B sulfanilamide (U. S. P. quality drug).

II. Drug as in I, wetted with 25 cc. of 1:1 acetone-alcohol. Then the solvent was evaporated on a steam bath and the drug powdered. This solvent was added as a control on the experiment described in Series III.

III. Drug as in I to which was added 0.5 per cent of stearic acid as an alcoholic-acetone solution. The solvent was then evaporated and the sample powdered as in II.

Each of the series was then divided into three portions, and each portion was treated as follows:

I*a*, II*a* and III*a*—stored in stock screw-cap bottles in a closet.

I*b*, II*b* and III*b*—exposed in open Petri dishes to ultraviolet light for a total of 25 hours.

I*c*, II*c* and III*c*—exposed in open Petri dishes to bright sunshine for a total of 25 hours.

A comparison of the samples after the exposures showed that their appearance, both by daylight and by ultraviolet, was qualitatively the same, although the changes effected by the exposures were not as well marked as those described in Table III. An explanation for this was seen in the fact that Brand B sulfanilamide did not possess the bright greenish yellow fluorescence of Brand A sulfanilamide, Brand B having only a faint brownish fluorescence. An examination of Brand C sulfanilamide showed that it possessed a bright, yellowish white fluorescence. Brand D sulfanilamide was a white crystalline sample. In ultraviolet light, it had a light cream color with a yellowish fluorescence.

When the results tabulated in Table II were compared with the appearance of the samples under ultraviolet light, the fluorescent characteristic of the drug appeared to be related to the small amount of residual "impurity" for, although the U. S. P. assay and the melting points of the four brands of sulfanilamide were found satisfactory, the amount and the melting characteristics of the ether soluble residues differed appreciably for each brand of drug. However, the relation of the impurities to the fluorescence was not clear since none of the ether soluble residues possessed this characteristic. All of them appeared dark brown in ultraviolet light. On the other hand, the exposure tests indicated that the amount of photochemical action was dependent upon the power of extraneous material as the addition of stearic acid materially increased the depth of the brown discoloration produced.

In order to determine whether the amount of ether soluble residue would be increased appreciably by photochemical decomposition, a second portion of 100 Gm. of Brand B sulfanilamide was first extracted with anhydrous ether, then dried. Three series of samples I, II and III of 30 Gm. each were then made as above. Each series was again subdivided into three 10 Gm. portions. A portion of each series was treated in one of the following ways:

I*a*, II*a*, III*a*—kept in a dark closet.

I*b*, II*b*, III*b*—exposed to 25 hours of ultraviolet light.

I*c*, II*c*, III*c*—exposed to 25 hours of sunshine.

After the exposures, each sample was once more subdivided into a reference (*R*) sample and an experimental (*E*) sample. The *E* samples were extracted with five 5 cc. portions of ether, the solvent evaporated, and the residues weighed. The average of three subsequent extractions with similar amounts of ether was then subtracted from the first weight in order to correct for the amount of sulfanilamide in the residue. In the case of Set III, an additional correction was made because of the added stearic acid. The results obtained are given in Table IV.

Table IV.—Ether Residues from Sulfanilamide Exposed to Ultraviolet and Sunshine

(Corrected for sulfanilamide and stearic acid content.

Exposure	I (Untreated) Per Cent	II (Acetone + Alcohol) Per Cent	III (15% Stearic Acid Added) Per Cent
(*a*) Closet	0.04	0.04	0.04
(*b*) Ultraviolet	0.04	0.10	0.11
(*c*) Sunshine	0.04	0.10	0.29

Table III.—Effect of Ultraviolet and of Sunshine on Sulfanilamide

Sample	Appearance in Daylight	Appearance in Ultraviolet Light
U. S. P. sulfanilamide	Clean white powder	White with a bright greenish yellow fluorescence
I*a* + 18 hrs. ultraviolet	Slightly discolored	Very light cream colored, slight fluorescence
I*b* + daylight + 24 hrs. sunshine	Light brown colored	Distinctly browned, no fluorescence
II*a* + stearic + 18 hrs. ultraviolet	Browned	Distinctly browned, no fluorescence
II*b* + stearic + daylight + sunshine	Distinctly browned	Dark brown, no fluorescence

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.

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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.