SCIENTIFIC SECTION

SOME OBSERVATIONS ON THE QUANTITATIVE DETERMINATION OF THE ANTHRAQUINONE DERIVATIVES IN CATHARTIC DRUGS.[1,2]

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These constituents of cathartic drugs are usually determined by extraction, followed by direct weighing, colorimetric determination or precipitation. Tschirch[4] and his students and Warin[5] have made the most elaborate study of these methods. Daels extracts the "free" anthraquinones by means of chloroform, and afterwards liberates the combined anthraquinones by hydrolysis with sulphuric acid in the presence of chloroform. The authors wish to present some observations made during the attempted determination of these constituents by Daels' method[6] and some of its possible modifications. The method as published is as follows:

Determination of Free Anthraquinones.—Place five grams of finely ground drug in a 350-cc. Erlenmeyer flask, add 200 cc. of chloroform and weigh the flask and contents. Connect the flask with a reflux condenser and boil gently for 15 minutes, cool, decant the solvent through a filter and wash the residue and filter with chloroform. Shake the solution with 5% sodium hydroxide until the color is removed from the chloroform and the alkali extracts are no longer colored pink. Shake the combined alkali extracts with chloroform to remove emulsified fat, then acidify the solution with hydrochloric acid and extract the liberated anthraquinones by repeatedly shaking with small portions of chloroform. Filter the chloroform extracts into a tared beaker and dry to constant weight at 100°.

Determination of Combined Anthraquinones.—Return all of the drug to the extraction flask and add chloroform to the previous weight. Add 50 cc. of 25% sulphuric acid, weigh again and heat under a reflux condenser at the boiling point of the chloroform for two and one-half hours. Cool and make up to the original weight with chloroform. Draw off 150 cc. of chloroform through a small filter into a separatory funnel and shake with 50 cc. of 10% sodium bisulphite solution. Draw off and reject the bisulphite solution and shake the chloroform with 100 cc. of 1% hydrochloric acid, drawing off and rejecting the acid. Finally evaporate the chloroform solution to constant weight at 100° in a tared beaker.

This method has been well commented on, and since it offers the simplest possible form of extraction procedure shows possibilities of developing into a fairly "fool proof" method. It has been made a subject for study by the Association of Official Agricultural Chemists, and the reports of the referee, Fuller,[7] show considerable progress in the study of the method. However, the results obtained by different operators upon the same lot of drug have shown that the variations are

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[1] Contribution from the Laboratory of Analytical Chemistry at the University of Illinois.

[2] Presented before the Scientific Section at the 73rd Annual Meeting, Des Moines, Iowa, August 24–28, 1925.

[3] This paper forms part of a thesis to be presented by M. C. T. Katti in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of the University of Illinois.

[4] Tschirch, *Pharm. Post,* Nos. 17, 19 (1904); *Arch. Pharm.,* 245, 151 (1907).

[5] Warin, *Jour. d. pharm.,* (6), 8, 253 (1905).

[6] *Bull. acad. roy. méd. Belg.,* (4), 27, 350 (1913).

[7] Fuller, *Jour. Assoc. Off. Agri. Chem.,* 5, 575 (1922); 7, 7 (1923).

probably due to more than the mere personal equation. In the first article by Fuller he reports the results of collaborating analysts where the variation between the averages of the individuals are as much as threefold.

In this article Fuller describes a colorimetric method said to have been developed by the Bureau of Internal Revenue.

The drug was hydrolyzed by boiling with 5% sulphuric acid under a reflux condenser for 15 minutes. The mixture, after cooling, was shaken repeatedly with ether until a portion gave no color with a trace of potassium hydroxide. The aqueous liquid was then warmed to expel ether vapors and again boiled for 15 minutes, cooled and extracted as before. The ether extracts were finally all combined, extracted with potassium hydroxide, this solution diluted and the color finally compared with that of a 1–1,000,000 solution of aloe-emodin in potassium hydroxide.

Fuller's second report presents a gravimetric and a colorimetric method for "total" anthraquinones, discarding the previous distinction between the "free" and "combined" derivatives.

For the gravimetric determination the drug was hydrolyzed by boiling with 25% sulphuric acid in the presence of chloroform and after separating the chloroformic solution the hydrolysis mixture was twice washed with chloroform. The combined chloroformic solutions were evaporated to one-third their original volume and shaken out with 10% sodium hydroxide. These extracts were combined, acidified with hydrochloric acid and shaken out with chloroform. The chloroform was then evaporated and the residue dried and weighed.

The residue of total anthraquinones was then dissolved as completely as possible in ether and the yellowish red solution matched in the Lovibond Tintometer against the red and yellow slides. An aliquot portion of the ether extract was then shaken with strong ammonia, the ether rejected and the color of the ammoniacal solution matched against the red slides in the tintometer.

Discrepancies of the same nature as appeared in the first paper are found when the reports of the collaborating analysts are compared.

EXPERIMENTAL.

The writers have analyzed a series of drugs according to Daels, with the following results.

TABLE I.—"FREE" AND "COMBINED" ANTHRAQUINONES, ACCORDING TO DAELS.

Drug.[1]	"Free" anthraquinones.	"Combined" anthraquinones.
Rhubarb, sample N	0.15	3.44
	0.16	3.28
Rhubarb, sample G	...	3.33
	...	3.18
Cascara, sample F	0.22	2.82
	0.25	2.81
Cascara, sample N	0.13	3.46
	0.14	3.33
Cascara, sample G	0.26	2.03
	0.19	2.13
	0.26	...
Senna, sample N	0.08	2.48
	0.08	2.57
Frangula, sample N	0.10[2]	4.09[3]
	0.13[2]	4.09[3]
	0.07[4]	3.52
	0.09[4]	3.49

[1] Samples designated N were milled at the College of Pharmacy of the University of Minnesota under the direction of Professor E. L. Newcomb. Sample F was a commercial lot purchased from Fuller, Morrison and Co., and milled in the author's laboratory. Samples G were commercial samples milled by Gilpin, Langdon and Company.

[2] The samples were digested with chloroform for two hours.

[3] The samples were hydrolyzed for six hours.

[4] The samples were digested with chloroform for thirty minutes.

A number of observations were made during these analyses.

1. The extraction with sodium hydroxide was slow, and much difficulty was encountered with emulsions. The rate of extraction was dependent upon the length of the period of shaking.

2. During the extraction with alkali the chloroform solution becomes turbid, the turbidity persisting in cold solution and disappearing on warming. Even when the separated sodium hydroxide solution is colorless the chloroform has a dirty brown color.

The sodium hydroxide extract on standing over night showed the separation of a colorless gelatinous precipitate, which at times occluded some of the pink alkali salt of the anthraquinone. On standing for several days the alkaline solution became paler in color and after a number of weeks lost entirely the pink color.

3. The final residues of both free and combined anthraquinones were slightly waxy. The residues of free anthraquinone could be brought to constant weight in one hour's time, the combined anthraquinones requiring about three hours.

Recovery of Pure Emodin.—Compared with the analyses of different specimens of these drugs as published by Gunton and Beal,[1] the values for free anthraquinones are very much lower. It was thought that this might be due to incomplete recovery of the anthraquinones by either the alkaline extraction or the subsequent extraction from the acidified extract.

Therefore, weighed quantities of pure emodin were dissolved in ether or chloroform and extracted by repeatedly shaking with small portions of 5.0% sodium hydroxide. The exhausted solvent was washed with a little water to remove retained alkali, evaporated to dryness and the residue weighed. The alkaline extract was then acidified with hydrochloric acid and extracted with chloroform. The aqueous solution still having a yellow color when the chloroform came away colorless, it was evaporated to dryness and extracted in a Soxhlet extractor with chloroform. The solvent was evaporated and the additional recovery determined.

TABLE II.—RECOVERY OF PURE EMODIN BY SHAKING-OUT PROCESS.

Wt. Emodin taken.	Solvent.	Residue.	Emodin first extracted.	% initial recovery.	Emodin second recovery.	Total recovery.	% total recovery.
0.1655	Ether	0.0004	0.1599	96.61	0.0050	0.1649	99.63
0.0916	Ether	0.0003	0.0860	93.88	0.0034	0.0894	97.59
0.1006	Ether	0.0001	0.0958	95.22	0.0020	0.0978	97.21
0.1058	Ether	0.0002	0.1020	96.40	0.0036	0.1056	99.81
0.1046	Chloroform	0.0007	0.1011	96.65	....	0.1011	96.65
0.1429	Chloroform	0.0005	0.1385	96.92	....	0.1385	96.92

1. Although emodin is much more soluble in ether than in chloroform, twelve extractions with 15-cc. portions of 5% sodium hydroxide were required for complete extraction of the emodin from 200 cc. of chloroform while only six extractions were necessary when the emodin was dissolved in 200 cc. of ether.

[1] Gunton and Beal, JOUR. A. PH. A., 11, 669 (1922).

2. The residue left in the initial solution after the alkali extraction does not give any color with alkali and is evidently not a hydroxymethyl anthraquinone.

3. The acidic solution always retains several milligrams of emodin which is only slowly given up to the immiscible solvent.

The Solubility of Emodin.—Before proceeding further with a study of "shaking out" methods it seemed desirable to obtain more data as to a suitable immiscible solvent. Since emodin is the anthraquinone derivative present in largest amount, and a supply of the pure material was available it was selected as the subject of this investigation.

Accordingly the selected solvent was boiled for two and one-half hours with an excess of pure emodin, allowed to stand at room temperature to attain saturation and filtered. A measured volume of filtrate was evaporated to dryness and brought to constant weight. The results obtained were as follows:

TABLE III.—SOLUBILITY OF EMODIN IN VARIOUS IMMISCIBLE SOLVENTS.

Solvent.	Grams of emodin per 100 cc. of saturated solution.
Carbon Tetrachloride	0.0102
Chloroform	0.0705
Benzene	0.0405
Carbon disulphide	0.0088
Ether	0.1400

Use of Ether as a Solvent.—In view of the greater solubility of emodin in ether and the more rapid extraction from this solution by alkali, an attempt was made to substitute ether for chloroform in the Daels' method.

The drug was refluxed with 200 cc. of ether for 30 minutes, then filtered and extracted with 5% sodium hydroxide. Five extractions were sufficient for complete removal of the anthraquinones. A waxy residue left upon evaporation of the ether did not give the Borntraeger reaction with alkali. Upon allowing the alkaline extract to stand for four to five hours some of the gelatinous precipitate previously mentioned appeared. This solution was acidified with hydrochloric acid and extracted with chloroform, which was evaporated and the residue weighed. Cascara, sample G, was thereupon found to contain 0.48% and 0.49% of "free" anthraquinones.

Nature of the Impurities in the Extract.—The material forming the gelatinous precipitate in the alkaline extract seems to be more soluble in ether than in chloroform. Therefore when the initial extraction is made with ether less of this material passes into the alkaline extract, and we may expect a more complete recovery of anthraquinone in the chloroform extraction since the possibility of occlusion by this precipitate will be lessened. Solution of the weighed anthraquinone residues in ether showed the presence of this material demonstrating that an erroneously high value for free anthraquinones is obtained. There is furthermore another substance in the original ether or chloroform extract which is extracted by the alkali to form a yellow to orange solution, depending upon the concentration.

The gelatinous precipitate was separated by repeated centrifuging and washing with water. At room temperature it is a soft solid waxy substance with a slight yellow color. At the temperature of the steam bath it turns brown. If during the extractions the system is heated, it dissolves readily in the chloroform or ether, separating again on cooling. The material once separated in the solid state is only slowly soluble in chloroform or ether, and no longer forms a colloidal solution with sodium hydroxide.

The substance yielding the yellow solution in sodium hydroxide is soluble in water, chloroform and ether, and tends to distribute itself between water and the immiscible solvent. The sodium hydroxide extract from the original solution when evaporated nearly to dryness on the steam bath, a little salt added to increase the surface and the drying completed, gave, when extracted with chloroform in a Soxhlet extractor, a turbid extract of yellow color. The extract gave no Borntraeger reaction with alkali, showing the absence of hydroxy methyl anthraquinones. When the residue in the extraction tube was taken up with water a purplish red turbid extract was obtained, showing that the substance forming the basis of the gelatinous precipitate was incompletely extracted.

Effect of Salting Out.—By saturating the sodium hydroxide solution of the hydroxy methyl anthraquinones with sodium chloride the sodium salts of these compounds were quantitatively separated as a pink precipitate. The precipitate could be separated by filtration, which was slow. When the precipitated sodium salts had their origin in the original drug extract, the filtrate was bright yellow in color, without the slightest pink tint. When the filtrate was acidified no change in color could be seen. Extraction of the acidified filtrate with chloroform gave a yellow extract yielding a yellow residue which dissolved in water to a yellow solution and showed only a light orange color upon addition of sodium hydroxide.

The pink precipitate upon the filter was dissolved by passing 0.1 normal sodium hydroxide through the filter until the solution came through colorless. The gelatinous matter remained entirely upon the filter as a brown residue. Upon acidifying the filtrate with hydrochloric acid and extracting with chloroform the anthraquinones were completely recovered. When the chloroform extract was evaporated to dryness the residue was free from oily matter and dissolved completely in sodium hydroxide to give a clear solution. It was also noticed that by using a comparatively large volume of ether for the original extraction of the drug and a weak solution of sodium hydroxide, not over 0.1 normal, for the shaking out, the quantity of the precipitating foreign material obtained was materially decreased. While according to all qualitative tests made the separations were apparently complete, the time required for solution of the sodium salt by washing the filter with aqueous alkali was such as to discourage further study of this scheme.

Variations in Daels' Procedure.—The "salting out" method being entirely too time consuming, Daels' method was tried with various modifications as to solvents, time of extraction and methods of clarification. In every case cascara, sample G, was the drug used. The percentages of "free" and "combined" anthraquinones as determined by these various modifications will be found in Table IV.

A. Five grams of drug was refluxed with 200 cc. of ether for one hour, cooled, filtered and washed with ether. The filtrate was shaken with 5% sodium hydroxide solution with intermediate washings with water. The combined alkali extract was acidified and extracted with chloroform. This extract was evaporated to dryness and dissolved in a few cc. of 5% sodium hydroxide. The gelatinous precipitate which formed was removed by filtration, and the solution acidified and extracted with chloroform. The residues so obtained were weighed as "free" anthraquinones. The final residues after weighing were dissolved in dilute sodium hydroxide, still yielding the gelatinous precipitate.

B. Five grams of drug were digested with 200 cc. of carbon tetrachloride for 3 hours. After filtration the solvent was distilled and the residue dissolved in 5% sodium hydroxide. A heavy precipitate was formed. After the mixture had stood over night it was filtered and the

filtrate acidified and extracted with chloroform. The chloroformic extract was evaporated to constant weight and weighed as "free" anthraquinones.

The residual drug was returned to the flask and heated for two and one-half hours with 200 cc. of carbon tetrachloride and 50 cc. of 25% sulphuric acid. After separating the solvent layer it was washed with a little water while the acid solution was washed with a little of the solvent, the washings being added to the principal extract. The solvent was evaporated and the residue taken up with sodium hydroxide solution, obtaining only a slight precipitate. This extract was acidified and shaken out with a mixture of chloroform and carbon tetrachloride. These extracts were combined and shaken with 10 cc. of a saturated solution of lead acetate. The lead solution was washed with a little of the solvent, then rejected. The organic solution was then evaporated and weighed as "combined" anthraquinones.

C. Two and one-half grams of drug were digested with 250 cc. of carbon tetrachloride for two and one-half hours and filtered after cooling. The solvent was evaporated, the residue taken up with a little sodium hydroxide solution and shaken with infusorial earth, then filtered. The filtrate was acidified and extracted with carbon tetrachloride, the solvent distilled and the residue weighed as "free" anthraquinones.

The drug was then returned to the flask and refluxed for two and one-half hours with 250 cc. of carbon tetrachloride and 50 cc. of 25% sulphuric acid. The carbon tetrachloride solution was separated and shaken with saturated lead acetate solution as above. The carbon tetrachloride was then evaporated and the residue weighed as "combined" anthraquinones.

D. Two and one-half grams of drug were digested as in the preceding experiment and the extract repeatedly shaken with sodium hydroxide solution. The alkali extract was filtered, then shaken vigorously with some infusorial earth, allowed to stand over night and again filtered. This filtrate was acidified and extracted with chloroform, the "free" anthraquinones being obtained by drying this extract. The "free" anthraquinones after the final weighing were again dissolved in chloroform and the solution shaken with lead acetate, but no precipitate was observed. "Combined" anthraquinones were determined exactly as in procedure C, with an additional purification by extraction with alkali, shaking with infusorial earth and extraction of the acidified solution with chloroform.

E. The procedures under "D" were repeated, using chloroform throughout in place of carbon tetrachloride. The residues obtained in following this procedure were brown, while those obtained in "D" were yellow with only a faint brown tint.

TABLE IV.—PERCENTAGES OF "FREE" AND "COMBINED" HYDROXYMETHYL-ANTHRAQUINONES AS DETERMINED BY THE VARIOUS MODIFICATIONS OF DAELS' METHOD, USING CASCARA, SAMPLE G.

Modification.	"Free" anthraquinones.	"Combined" anthraquinones.
Daels' Method	0.26	2.03
Daels' Method	0.26	2.13
Daels' Method	0.19	....[1]
A	1.34	....
A	1.29	....
B	1.93	1.05
B	1.95	1.06
C	1.65	1.00
C	1.50	0.92
D	0.29	0.74
D	0.25	0.75
D	0.21	0.64[2]
D	0.22	0.67[2]
E	0.15	0.88
E	0.13	0.96
F	0.34	2.16 (0.99)[3]
F	0.36	2.16 (1.00)[3]

[1] Determinations were not completed in the spaces so indicated.

[2] The final extraction was made with carbon tetrachloride instead of chloroform.

[3] The values in parentheses are the purified anthraquinones.

F. Procedure "E" was followed except that in determining "combined" anthraquinones the chloroformic solution was evaporated to dryness and the residue weighed after the lead acetate treatment. These values were reported as "crude, combined" anthraquinones. The residue was then dissolved in chloroform and the procedure completed as in "E." These values were then reported as "purified combined" anthraquinones.

Analysis of Fluidextract of Cascara.—A quantity of fluidextract of cascara was prepared in the laboratory according to the procedure in the U. S. P. IX and analyzed as follows. Five cc. of the fluidextract was mixed with 250 cc. of chloroform and 50 cc. of 25% sulphuric acid, the mixture weighed and refluxed for two and one-half hours, then made up to the original weight with chloroform. The chloroform layer was drawn off with the aid of a separatory funnel and shaken with 50 cc. of lead acetate solution in a second funnel, a considerable amount of precipitate being formed. The chloroform solution was extracted by shaking with sodium hydroxide solution, and the combined alkaline extracts acidified and extracted with chloroform. The extracts were dried and weighed as "total" anthraquinones. These will be referred to in Table V as method "G." The procedure was repeated with the same fluidextract, using carbon tetrachloride in place of chloroform for the initial digestion. This will be known as method "H."

TABLE V.—ANALYSIS OF FLUIDEXTRACT OF CASCARA, U. S. P. IX.

Method.	"Total" anthraquinones.
G	0.88
G	0.85
G	0.88
G	0.81
H	0.54
H	0.60
H	0.56
H	0.55

An attempt was made to check these values by a colorimetric determination, dissolving the residues in sodium hydroxide solution. Pure emodin dissolved in sodium hydroxide could not be used as a comparison standard since a difference in tint was obtained, the residues from the drug showing a yellow tint through the red in the colorimeter. If one of the residues was taken as the standard and the other three members of the set compared therewith, the values checked throughout. However different color values were obtained between series G and H, making it impossible to use a residue from one series as a standard for the other.

DISCUSSION OF RESULTS.

The precipitate settling from the sodium hydroxide extract is not soluble in cold chloroform. The chloroform solution while being extracted becomes turbid while ether remains clear. The precipitate is apparently in colloidal form in chloroform. The precipitate forming in the sodium hydroxide solution from the ether extract upon standing seemed to vary inversely with the volume of ether. When the volume of ether solution was as low as 50 cc., the precipitate seemed to form immediately, three layers being formed in the system: the bottom, sodium hydroxide with a colloidal suspension of the gelatinous material, in the middle a thin layer of precipitate, and above the layer of ether.

The residue weighed as anthraquinones when dissolved in sodium hydroxide usually yielded a white gelatinous precipitate. Furthermore the yellow non-anthraquinone material is distributed between the ether and the sodium hydroxide, with little difference in color due to either medium.

In the analysis of the various drugs according to the method of Daels or the modifications as used herein, a comparison of the weights of mixed anthraquinones reported as being in the "free" and "combined" forms with the weight of emodin which will dissolve in the volume of solvent used is illuminating.

TABLE VI.—WEIGHTS OF ANTHRAQUINONES EXTRACTED COMPARED WITH THE MAXIMUM AMOUNT OF EMODIN WHICH COULD BE DISSOLVED.

	Drug.		Free anthraquinone, Gm.	Combined anthraquinone, Gm.	Solubility of emodin in volume of solvent used, Gm.
Daels'	Rhubarb	N	0.0080	0.1720	0.1410
"	Rhubarb	G	...	0.1665	0.1410
"	Cascara	F	0.0110	0.1410	0.1410
"	Cascara	N	0.0070	0.1730	0.1410
"	Cascara	G	0.0130	0.1065	0.1410
"	Senna	N	0.0040	0.1285	0.1410
"	Frangula	N	0.0065	0.1760	0.1410
				0.2045	
Method A	Cascara	G	0.0670		0.2800
Method B	Cascara	G	0.0965	0.0525	0.0204
Method C	Cascara	G	0.0412	0.0250	0.0255
Method D	Cascara	G	0.0073	0.0188	0.0255
Method E	Cascara	G	0.0038	0.022	0.1763
Method F	Cascara	G	0.009	0.064	0.1763

Unfortunately we have no data of sufficient accuracy on the solubility of the other anthraquinone derivatives which are present in these drugs. The assumption is made, therefore, that their solubilities are of the same order as that of emodin. We can accordingly account for the percentages of these derivatives as extracted by crediting them with mutual solvent effects upon each other, which effects are additive to those of the solvent liquid, or grant that non-anthraquinone material accompanies the extracted anthraquinones. This last is demonstrated in the presence of the substance forming the gelatinous precipitate and the yellow substance, both previously described.

Attempts to obtain a purer form of anthraquinone residue by shaking the ether or chloroform extract with decolorizing carbon resulted in absolute failure, the anthraquinones being entirely adsorbed by the carbon. Shaking the chloroform or carbon tetrachloride extracts with lead acetate solution was no more efficient as a means of purification, since it also failed to remove the cause of the gelatinous precipitate.

The largest amount of the non-anthraquinone material is obtained with the residue of "combined" or "total" anthraquinones. This has lead to the idea that it is largely a product of hydrolysis, the parent substance accompanying the anthraquinone derivatives in the drug. The drug after percolation with hot water, as in the preparation of the fluidextract, is not devoid of anthraquinones. It does not, however, yield any of this contaminating material which is precipitated by lead acetate showing that it will be concentrated in the fluidextract. The yellow waxy material will still be found in part in the extracted drug.

The presence of the resinous or gelatinous material previously described will probably lead to unduly high values for "free" anthraquinones. The extent of the error increases with the length of the initial digestion. If this period is shortened to decrease the error, it results in an increased error in the "combined" anthraquinones, as this resinous material will then appear in the second extract, from which it is apparently not completely removed by treatment with sodium bisulphite, lead acetate, or kieselguhr. These obstacles probably existed, although possibly unrecognized, in the applications of Daels' method by Fuller and his collaborators.

The most serious objection to the tintometer method as described by Fuller is that in measuring the yellow color of the ether solution no account is taken of the presence of the yellow non-anthraquinone material. It would be actually possible in this way to obtain a positive value on material free from anthraquinone. Where this determination is afterwards checked colorimetrically by the alkali salt method, the difficulty is avoided in part, but the presence of this yellow material now in the water solution, does not contribute to a simplification of the colorimetric method.

Neither does it seem proper to use as a colorimetric standard a solution of aloe emodin for comparison with solutions the principal color of which is due to frangula emodin. We are of the opinion that the only satisfactory color standard can be obtained from a specimen of the species of drug under examination from which the anthraquinones have been extracted and determined gravimetrically with the greatest quantitative accuracy possible. The residue so obtained might be dissolved in alkali and used as the basis of a color standard.

Finally attention should be called to the various published analyses of drugs of this class, including those reported in this paper in which will be noted the excellent agreement between duplicate determinations, although they may be out of all bounds of reason as solubility data is considered. The extraction and removal of non-anthraquinone material is very evidently largely influenced by certain physical conditions which are established by each operator in his duplicate analyses.

The authors are continuing this work, first, with a view to establishing conditions which will lead to accuracy of results regardless of the time involved, and then to an elimination of procedure by which the method may be sensibly shortened without an appreciable loss in accuracy.

NOTES ON REDUCED IRON.*

BY GEORGE L. KEENAN.

Some time ago it was suggested to the writer that a comparative microscopical study of products appearing on the market as reduced iron or iron by hydrogen would be of interest in determining any diagnostic microscopical differences that might exist in the products, thereby eventually aiding in differentiating any non-pharmacopœial articles from the pharmacopœial. The purpose of this paper is to call attention to these differences.

Reduced iron is formed by the action of hydrogen upon ferric oxide, resulting in a product which should contain, according to the U. S. P. X (abstract), not less than 90 per cent of metallic iron (Fe). In general appearance the resulting product should consist of an odorless, grayish black, lusterless powder, which should pass through a No. 100 sieve. As an additional test of its genuineness, the Pharmacopœia stipulates that when 1 Gm. of reduced iron is heated in a porcelain crucible with a small Bunsen flame until a bluish black color appears without glowing, the particles of the material should glow brightly as they fall through the air when poured from the crucible.

* Scientific Section, A. PH. A., Des Moines meeting, 1925.