amount of esterification that has taken place in the sample.

7. That barium ethyl sulfate is not hydrolyzed in dilute slightly acid aqueous solution, to any appreciable extent.

In conclusion, I wish to give credit to J. M. Humble for his assistance to me in conducting the experimental part of this work.

KENTUCKY AGRICULTURAL EXPERIMENT STATION.

A METHOD FOR ASSAYING SPIRITS OF CAMPHOR.

By ARTHUR T. COLLINS.

Received February 29, 1912.

Spirits of camphor can be assayed, and the percentage of camphor very accurately determined, by the use of the polariscope. It has been long known that camphor is optically active, some camphors rotating the plane of polarization to the right, and some to the left. The amount of this deviation will vary in camphors coming from different localities, or even from the same locality.

Most camphors are dextro gyrate, a 10 per cent. solution giving in a 200 mm. tube an angle of 8°, more or less. In order to know the percentage of camphor in a solution, it will be necessary to know the rotation angle of the particular camphor that was used in making up the solution. This can be done by taking 50 cc. or more of the spirits of camphor, and evaporating on a water bath; by this operation the camphor can be obtained free from alcohol or moisture. Of course there is a considerable loss of camphor, often as much as 30 to 40 per cent. but the camphor that remains has exactly the same rotation as the original.

The camphor obtained by evaporation on the water bath contains all the non-volatile impurities that were in the original solution, and in order to get rid of these, the camphor is transferred to a watch glass of about 7 or 8 cc. diameter, having for a cover a glass funnel of the same diameter—both funnel and watch glass having ground edges. The apparatus is placed over a low flame, and the camphor sublimed on to the funnel.

We have now the pure camphor that went to make up the original solution. This is made up to a 10 per cent. solution in a 25 cc. volumetric flask, and used as a standard for the determination of the camphor in the spirits of camphor in question. The method is very accurate and the entire operation may be accomplished in two hours time.

Using camphors from different sources made up to 10 per cent. solutions, the results are as follows:

	Rotation in minutes.	Per cent.
Sample A.....	482.8	9.94
Control.....	485.3	
Sample B.....	488.5	10.003
Control.....	488.3	
Sample C.....	492.5	10.01
Control.....	491.7	

If the original gum camphor has been impure, or if optically active substances have been added to increase the rotation, these may be found on the watch glass, after the camphor has been sublimed, and can then be weighed or investigated.

In case sugar has been added, this will be found by evaporating in a drying oven at a temperature of 105° C. After all smell of camphor is gone the sugar should be made up to volume with water, and the amount of rotation deducted from the optical rotation of the original spirits.

The entire method is as follows:

Polarize in a 200 mm. tube, making correction for temperature by adding 1/2 minute for every degree C. above 20° C., and deducting same amount below 20° C. Take the mean of 10 readings, place 50 cc. or more of the spirits in an evaporating dish on water bath, using cold air jet if convenient, to facilitate evaporation. Just before the camphor solidifies, stir with glass rod, so that it may become dry as soon as possible. When quite dry place on watch glass having funnel cover, over a low flame, with wire gauze between, and fitting closely to the bottom of the watch glass.

When the camphor is entirely sublimed, remove the pure camphor from the funnel, take 2 1/2 grams of this, make up with alcohol in a 25 cc. flask, polarize as in the first case; this is called the "control." The percentage is found by dividing the minutes of rotation of the control into minutes of rotation of the original sample, multiplied by ten.

SWARTHMORE, PA.

SUGGESTED MODIFICATIONS TO THE U. S. P. ASSAY OF OPIUM.¹

By R. NORRIS SHREVE.

Received January 17, 1912.

The Pharmacopoeia directs for the determination of Morphine, an aqueous extraction of the opium, concentration of these extracts, precipitations of the morphine from a weak alcoholic solution, and determination of the impurity in the precipitated morphine by weighing of the substance insoluble in lime-water. As thus carried out the method gives results that are not accurate. There are the following sources of error:

1. Incomplete extraction of the opium.
2. Retention of morphine by the mother liquor during precipitation.
3. Inaccuracies in the lime water method for determination of the impurities in the precipitated morphine.

INCOMPLETE EXTRACTION OF THE OPIUM.

The Pharmacopoeia directs for the extraction: Agitation every ten minutes (or continuously in a mechanical shaker) during three hours, followed by filtration, washing, re-agitation, and re-washing. In case of certain opiums this procedure will not exhaust the opium of its morphine as is shown in Table I.

Comment on Table I.—The three samples of opium were of Turkish grade and of a moderately fine powder. In every case ten grams were used for assay. Instead of determining the purity of the crude morphine by the lime water method as the U. S. P. directs, the more accurate but slightly longer Mallinckrodt

¹ Presented at the A. C. S. Meeting at Washington, D. C., December, 1911.

TABLE I.

No.	Outline of extraction, U. S. P. Method except as indicated.	Weight of crude precipitated morphine. Grams.	Purity of crude by Mallinckrodt re-assay. ¹ Per cent.	+ correction for solubility of morphine in U. S. P. mother liquors. Grams.	Pure morphine. Per cent.
Opium sample 1.					
1	In mechanical shaker for 8 hrs.	1.5875	94.6	0.052	18.79
	Marc of preceding reextracted.	0.3185		0.024	
2	Extracted 3 times in shaker.	1.909	93.0	0.0635	18.39
3	Opium triturated in mortar, then put in shaker; marc of preceding re-extracted.	1.6265			18.39
		0.125			
4	Extracted shaker; Lamar ² modification applied	1.3125	94.6	0.060	13.02
5	Opium + 45% lime, extracted, acidified; Lamar modification applied	2.097	88.7	0.055	19.16
Opium sample 2.					
6	In mechanical shaker for 8 hours.	0.988	90.6	0.050	9.45
7	Duplicate.	1.030	91.8	0.51	9.96
8	In very rapid shaker for 12 hours.	1.623	89.3	0.060	15.11
Opium sample 3.					
9	In mechanical for 8 hours.	0.914	91.3	0.051	8.86
10	Duplicate.	0.960	91.1	0.051	9.26
11	In very rapid shaker for 8 hours.	1.668	89.6	0.052	15.46
Opium sample 4.					
12	In mechanical for 8 hours.	0.987	93.7	0.051	9.76
13	Duplicate.	0.990	91.4	0.051	9.56
14	In very rapid shaker for 8 hours.	16.55	90.6	0.052	15.51

¹ Proc. A. O. A. C. 23rd convention, Bur. Chem., *Bull.* **105**, 128 (1906).² *Am. J. Pharm.*, **71**, 36 (1900).

re-assay was used. This re-assay is carried out as follows:

Morphine up to 2 grams is mixed with 0.5 gram of freshly slaked lime, and 18 cc. of water added, rotated occasionally during half an hour, filtered without suction (suction causes foaming), and washed with lime water until the filtrate weighs 35 grams; 3 cc. of alcohol added, flask rotated; 20 cc. of ether added, flask rotated again; finally 0.5 gram of ammonium chloride added, and the flask shaken for ten minutes; set aside for 2 hours (or over night) for the morphine to precipitate. Morphine filtered and washed, in the manner which the U. S. P. directs in case of the crude morphine. Correction for the morphine soluble in alcoholic mother liquors is determined by blank assay on pure morphine. The "+ correction for solubility of morphine in U. S. P. mother liquors" was obtained likewise by running a blank precipitation, but of course under the conditions parallel to the U. S. P. assay.

The opium of sample 1 was not completely exhausted of its morphine by the aqueous extraction, as is readily seen from Experiments 1 to 4 of Table I. However, in Experiment 5, which was a lime assay, the opium was exhausted as determined by test on the marc. The details of this experiment are as follows: 10 grams opium were mixed with 4.5 grams of lime, and triturated with 100 cc. water for several hours, filtered and washed with water sufficient to make filtrate measure 150 cc.; retrituated and re-washed; filtrate acidified with dilute hydrochloric acid, concentrated, and inorganic matter, etc., thrown out by alcohol (Lamar Modification, *Loc. cit.*); alcohol evaporated and morphine precipitated as U. S. P. directs.

The opium of samples 2, 3 and 4 was exhausted of only about $\frac{2}{3}$ of its morphine by gentle mechanical shaking for 8 hours. By employing a mechanical shaker making three strokes a second, it was possible to exhaust the opium.

Such being the case with some opiums, I always

test the marc for completeness of extraction; for this, the following test is satisfactory: Wash the marc with an additional 100 cc. of water; evaporate the filtrate to about 2 cc., filter, add 4 cc. of washed ether, and ammonia to faint alkalinity. If the morphine was not all exhausted in the regular extraction, it will precipitate here in well defined crystals which give the characteristic color reactions. The precipitation is facilitated by scratching the inside of the test tube with a glass rod. I suggest that opium always be tested for completeness of extraction of its morphine, and that the Pharmacopoeia Revision Committee embody some such test as the one just given in the official method, with directions to repeat the assay using more vigorous shaking and more washing in case the test shows morphine in the marc. This repeating will be generally necessary, as by the time the result of the test is obtained the morphine in the assay will have been precipitated, since it is not worth while to wait for a test which will indicate morphine in the marc in only an occasional opium.

In case of the U. S. P. powdered opium, the substances used by the manufacturers to standardize the opium often retard the extraction of the morphine, so that in assay of opium of this kind, it is especially necessary to apply tests to determine completeness of extraction. Because of this difficulty of extracting some opiums, an aliquot part method for the assay of this drug is not advisable as one could never be sure of the relative amount of morphine in that part taken for assay.

RETENTION OF MORPHINE BY THE MOTHER LIQUORS DURING PRECIPITATION.

It is well known that the alcoholic mother liquors employed during the precipitation of the morphine hold in solution some of the morphine. This has been pointed out by many workers especially in the co-operative work on opium assaying, done during the leadership of the Bureau of Chemistry.

The amount of pure morphine retained by solvents present when the morphine is precipitated in the

U. S. P. assay is easily obtained by dissolving one and one-half grams of morphine in 5.5 cc. *N/1* sulfuric acid and water sufficient to weigh 20 grams; then precipitate under the conditions of the U. S. P. Table II gives results of such blank precipitations.

TABLE II.

No.	Amt. of morphine taken. Grams.	Amt. of morphine recovered. Grams.	Amt. of morphine retained by mother liquors. Gram.	Temperature during precipitation. Centigrade.
1	1.500	1.459	0.041	0°
2	1.500	1.443	0.057	20°
3	1.500	1.428	0.072	26°
4	1.500	1.426	0.074	26°

Comment on Table II.—In case of No. 4, instead of dissolving the morphine in 5.5 cc. of normal acid, there was used 16.5 cc. to ascertain if the decrease of free ammonia and the increase of ammonium salts would have an appreciable effect on the morphine retained; the result shows no effect. The length of time for precipitation was over night, though for the blanks themselves one or two hours is sufficient.

The corrections for solubility of morphine in Table I were determined by such blanks run parallel to the opium precipitation. There is no doubt, however, that the extractive matter in opium has an influence on the amount of morphine thus retained. To obtain some evidences on this point the following experiments were conducted:

Two assays were run: No. 1 on 10 grams of opium in the regular way; No. 2 on 100 grams of opium, using 10 times the quantities of solvents, etc. Both assays were precipitated under the same conditions: No. 1 gave 1.624 grams of crude morphine, reassaying 80.2 per cent. pure and, hence containing 1.302 grams pure morphine; No. 2 gave 16.007 grams of crude morphine, re-assaying 81.1 per cent. pure, and hence contained 13.0 grams pure morphine. Thus both gave the same percentage of morphine.

The mother liquors of No. 2, the 100 grams assay, were analyzed for morphine by this method: Made slightly acid, evaporated, mixed with excess of slaked lime and the cake exhausted of its morphine. The lime water filtrates were extracted with benzole to remove non-phenolic alkaloids, then acidified, evaporated to small volume, alcohol added to throw out inorganic matter, filtrates plus washings heated to drive off alcohol. Now morphine completely extracted with amyl alcohol, after making alkaline; amyl alcohol extracted with 10 per cent. sulfuric acid, and morphine precipitated by ammonia. The purity of this morphine was determined by Mallinckrodt re-assay: result, 1.15 grams of morphine alkaloid which remained dissolved in the mother liquors. This corresponds to 0.115 gram for regular U. S. P. mother liquors. A parallel blank on pure morphine indicated a correction of 0.069 gram, so it is apparent that in this case the extractive plus the solvent present held back considerably more morphine than is represented by the dissolving power of the solvents themselves.

This analysis of the U. S. P. mother liquors was very tedious, yet it should be carried on the mother liquors of assays from a number of different opiums before we can say just how much morphine is retained in the precipitation mother liquors. The

chloroform alcohol method of Eaton¹ could probably be used and would be simpler.

Of course it would be better to use an immiscible solvent method for the assay of morphine and thus avoid altogether this retention of the morphine in the mother liquors of the U. S. P. assay. I have successfully used an amyl alcohol extraction, but the method is tedious and long. It gives results agreeing with the modified U. S. P. method as was used for the assays of Table I.

The chloroform alcohol extraction method of Eaton looks very promising, but I would suggest, because of the difficulty in extracting opium, that instead of an aliquot part extraction of the opium, a complete lime extraction be employed. This indeed works very nicely, and to carry it out I mix with the opium 40 per cent. of its weight of lime and extract with lime water. Furthermore it would be advisable to collect a sufficient quantity of the morphine from the evaporation of the chloroform alcohol, and compare the purity by titration against the accurate Mallinckrodt re-assay (run with blank to determine morphine in the mother liquors).

INACCURACIES IN THE LIME WATER METHOD FOR DETERMINATION OF THE IMPURITIES IN THE PRECIPITATED MORPHINE.

These inaccuracies were pointed out by Kebler in the above-mentioned coöperative work under direction of the Bureau of Chemistry. My experience has been that the lime water method gives results that are from 3-7 per cent. higher than the truth, the error varying with the nature and amount of impurity with morphine. This variation in the impurity depends on the sample of opium and the treatment it has been subjected to, such as temperature during and upon the conditions of the assay.

The only satisfactory method for the determination of the purity of crude morphine in the U. S. P. and similar assays is the Mallinckrodt re-assay. Two objections have been brought against this method, namely (a) time involved, (b) correction for morphine in mother liquors.

In the hands of several chemists, whom I have observed, the extra time necessary over that required by the lime water method is the 15 or 20 minutes it takes to filter and wash the precipitated morphine from the re-assay. On the other hand it is neither essential to use counterbalanced or tared filter papers for filtration of the crude morphine, nor is it worth while to dry the crude morphine. After washing the crude morphine, suck it nearly dry, and then remove it to a small Erlenmeyer flask for solution in excess of lime, and filter through the same filter paper.

The corrections for morphine dissolved in the re-assay mother liquors can readily be obtained from blanks on pure morphine. This correction varies with temperature and slightly with individual manipulation. After a series of corrections for the conditions are determined, it is no longer essential to run a blank as the variations under ordinary conditions are relatively small. Table III gives a series

¹ *Bur. Chem., Bull.* 137, 188 (1910).

of blanks for different temperatures. The morphine used was purified by twice crystallizing from Columbian spirits and the alkaloid obtained by precipitating a solution of sulfate of morphine.

TABLE III.

No.	Amt. of morphine. Grams.	Amt. of morphine recovered. Grams.	Amt. of morphine retained by mother liquor. Gram.	Temperature during precipitation. Centigrade.
1	1.500	1.482	0.018	17°
2	1.500	1.481	0.019	22°
3	1.500	1.4745	0.0255	27°
4	1.500	1.474	0.026	29°

CONCLUSION.

We have discussed (1) the difficulty of completely extracting morphine from opium; (2) the amount of morphine retained by the alcoholic mother liquors of the U. S. P. method; (3) the determination of the purity of the crude precipitated morphine.

It is suggested (1) that a test always be applied to determine the completeness of the removal of the morphine; (2) that a correction be added for morphine dissolved in the mother liquors of the U. S. P. opium assay, and that provisionally a blank on pure morphine be made to determine this correction; (3) that the Mallinckrodt re-assay be used to determine the purity of the crude morphine.

It gives me pleasure, in connection with this paper, to acknowledge my indebtedness to the Lamar Chemical Works and to the Mallinckrodt Chemical Works.

LAMAR CHEMICAL WORKS,
NEWARK, N. J.

A NOTE ON THE DETERMINATION OF THE DIGESTIVE VALUE OF PAPAIN.

By J. R. RIPPETOE.

Received January 11, 1912.

H. T. Graber in a recent article (THIS JOURNAL, Dec., 1911, p. 919) gives a method for determining the digestive value of papain, using fine ground steak in 0.3 per cent. hydrochloric acid solution and comments as follows: "Some manufacturers claim that the test should be conducted in an alkaline media and that egg albumen should be used instead of raw beef; but to me this seems as absurd as to claim that pepsin should be tested upon starch in an alkaline media."

For several years this laboratory has employed a method for determining the digestive value of papain using egg albumen in an alkaline media. It might be of interest to note some results obtained by this method.

Method.—Prepare egg albumen as directed under pepsin assay U. S. P. 8th revision. Introduce into a 4-oz. wide-mouth flint bottle 40 cc. of a 0.1 per cent. sodium hydroxide solution and add 10 grams of the disintegrated albumen; stopper the bottle and shake vigorously until the albumen is broken up. Then add the papain in fine powder and mix by shaking gently for 15 seconds. Place the bottle in a water bath previously heated to 52° C. and digest at this temperature for 6 hours, removing the bottle every 10 minutes and shaking gently for 15 seconds. At the end of this period transfer the mixture to a 100 cc.

graduated stoppered cylinder, rinse the bottle with water, add the rinsings to the mixture and make the volume up to 70 cc. with water. Set the cylinder aside and after standing for 1 hour read off the volume of the deposit. The deposit may be read a second time after standing 16–18 hours (over night) which seems to give more positive results, especially if the volume is large.

The results of the assay of five samples by the above method are recorded in the following tables:

TABLE I.
Deposit read after standing

Sample.	1 hr.	16 hrs.
	Cc.	Cc.
Blank.....	52.0	43.0
A 0.1 gram papain.....	26.0	18.0
A 0.2 gram papain.....	8.0	8.0
B 0.1 gram papain.....	41.0	31.0
B 0.2 gram papain.....	36.0	18.0

The blank consisted of 10 grams of the disintegrated albumen in 40 cc. of the alkaline solution without the addition of papain and was carried through the digestion and agitation in the same manner as the tests containing the papain.

Sample A was assayed in an acid media using 0.2 grams of papain in fine powder with the results as shown in Table II:

TABLE II.
Deposit read after standing

	1 hr.	16 hrs.
	Cc.	Cc.
0.1 per cent. HCl solution.....	16.0	20.0
0.2 per cent. HCl solution.....	30.0	30.0
0.3 per cent. HCl solution.....	32.0	30.0

TABLE III.
Deposit read after standing

	1 hr.	16 hrs.
	Cc.	Cc.
C 0.1 gram papain.....	15.0	12.0
C 0.2 gram papain.....	3.0	5.0
D 0.1 gram papain.....	25.0	23.0
D 0.2 gram papain.....	13.0	12.0

TABLE IV.
Deposit read after standing

	1 hr.	16 hrs.
	Cc.	Cc.
C 0.1 gram papain.....	2.0	3.0
C 0.2 gram papain.....	0.8	1.0
E 0.1 gram papain.....	5.0	5.5
E 0.2 gram papain.....	1.0	1.5

It might be said that Sample A, Table I, had twice the digestive value of Sample B, since the deposit from 0.1 gram A was the same as the deposit from 0.2 gram B, reading after 18 hours.

The results shown in Table II indicate that some digestion takes place in 0.1 per cent. HCl but 0.2 or 0.3 per cent. inhibits the action of the ferment.

The variation in the volume of the deposit obtained with Sample C, as shown in Tables III and IV, is possibly due to the varying composition of the albumen of the eggs used, and suggests the advisability of using a standard sample of papain for comparison when making an assay of an unknown sample upon egg albumen.

The method is also applicable for determining the digestive value of pineapple juice. A sample of dry