LABORATORY NOTES.*

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NOTE ON THE ASSAY OF LOBELIA PREPARATIONS.

As the alkaloid lobeline found in lobelia is volatile, it is customary in the assay of this drug to obtain the alkaloid by macerating with a mixture of alcohol, ether, and ammonia water, shaking out an aliquot part with 2 percent sulphuric acid, making the acid extractions alkaline with sodium carbonate and shaking out with chloroform. The chloroform extractions are placed in a tared crystallizing dish containing 30 Cc. of ether saturated with hydrochloric acid gas. The whole mixture is then evaporated on a steam-bath and dried in an air-bath to constant weight. The residue of alkaloidal hydrochloride is then dissolved in water and titrated with decinormal silver nitrate. Difficulties were experienced in attempting to titrate the hydrochloride directly with silver nitrate, using potassium chromate as indicator. It was found, however, that quite concordant results with excellent end points can be obtained by using the Volhard method of titration.

NECESSITY FOR TREATING ALKALOIDAL RESIDUES WITH ETHER BEFORE DRYING TO CONSTANT WEIGHT.

It has long been recognized as desirable to treat all alkaloidal residues obtained by chloroform with ether before attempting to dry to constant weight, owing to the tenacity with which such residues retain chloroform.

A recent illustration of this fact was shown in an attempt to assay tablets of apomorphine hydrochloride $^1/_{10}$ grain. The method employed was to dissolve 30 tablets in 10 Cc. of water, render alkaline with sodium bicarbonate and shake out with chloroform. When the chloroform extractions were evaporated and dried to constant weight at 80° C. an excess of about 17 percent of alkaloidal salt was invariably indicated. An investigation of the purity of the materials used and of the actual quantities used in the manufacture having shown no error, attention was again directed to the assay process with a result that when the alkaloidal residue was treated with ether prior to drying to constant weight at 80° C., a concordant result of 99 percent of the required amount of apomorphine hydrochloride was obtained.

NOTES ON THE ASSAY OF FORMALDEHYDE.

The U.S.P. in the assay of formaldehyde directs to allow the formaldehyde, hydrogen peroxide, and normal alkali to stand for thirty minutes after the foaming has subsided.

This has often been proven to be too short a time for complete oxidation of the formaldehyde to formic acid. A far better plan, and one which we have adopted in this laboratory, is to allow the mixture to stand with frequent shaking until no more gas bubbles are noticed on shaking, which occurs after an hour or two. This longer standing cannot be objected to on the score of any methyl alcohol, which

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is usually present, being oxidized to formaldehyde and then to formic acid. In experiments using 0.5 Cc. of methyl alcohol, which is far in excess of that likely to be present, practically no standard alkali was used up even after forty-eight hours. Two lots of commercial formaldehyde solution which were examined recently for methyl alcohol contained 6.9 percent and 9.5 percent respectively.

There is some objection to longer standing, however, when acetanilide is present in the hydrogen peroxide used, because the acetanilide is gradually saponified and uses up some alkali. Several blank experiments in which the standard alkali and hydrogen peroxide contained $^3/_{16}$ grain of acetanilide per fluidounce were let stand for an hour and showed no loss of alkali, but after about forty-eight hours 0.4 Cc. normal alkali was used up. This is not a serious objection, as in the time required by the improved method the loss of alkali due to reaction with the acetanilide would be negligible. Furthermore, about 50 Cc. normal alkali is used in the assay and 0.4 Cc. is less than 1 percent of 50 Cc., so that the maximum loss of alkali and consequent gain of formaldehyde would be about 1 percent and fairly within the limits of experimental error common to a conditional method of this character.

If it is desired to completely overcome the slight error introduced by long standing a blank correction can be employed.

THE TIME REQUIRED BY RENNIN FOR COAGULATION OF MILK IS INVERSELY PROPORTIONAL TO THE AMOUNT OF RENNIN EMPLOYED.

In our present method of assay we determine the coagulation power of rennin at a fixed time for coagulation of seven and one-half minutes. It often happens that in a series of determinations on a sample of rennin of unknown strength none of the tests will show coagulation at exactly the expiration of seven and one-half minutes. It is a time consuming operation to repeat experiments until the coagulation is made to take place in exactly seven and one-half minutes. A large number of experiments were run to determine whether or not the coagulation can be calculated to a seven and one-half minute basis by inverse proportion when the actual coagulation time has been found to differ from seven and one-half minutes. As a result of these experiments it has been determined that it is indeed possible from weight of rennin used, weight of milk employed and time required for coagulation, to calculate the coagulating power by inverse proportion to a seven and one-half minute basis with a sufficient degree of accuracy for all practical purposes. As an illustration, Experiment No. 7 on rennin sample No. 8 is quoted as follows:

Time for coagulation.	Coagulating power on seven and one-half minute basis.
5½ minutes	1–12,000
7 minutes	1–13,400
7½ minutes	1–12,500
8½ minutes	1–13,200
9½ minutes	1–14,800
10 minutes	1–12,500

The average of these six different times for coagulation calculates to 1 : 13,000 coagulation power on a seven and one-half minute basis, which result is very close to the 12,500 obtained when the time of coagulation was exactly seven and one-half minutes.

Our regular method for the assay of rennin has been modified by the adoption of the following appended note:

When coagulation does not occur in exactly seven and one-half minutes calculation can be made by inverse proportion to a seven and one-half minute basis. For example, if 2½ Cc. of the prescribed rennin solution coagulates the milk in six minutes instead of seven and one-half minutes, then $6:30{,}000::7\frac{1}{2}:x$ ($x=37{,}500$). Likewise, if 2½ Cc. of the rennin solution coagulates the milk in eight and one-half minutes instead of seven and one-half, then the proportion becomes $8\frac{1}{2}:30{,}000::7\frac{1}{2}:x$ ($x=26{,}470$).

The above calculation can be resorted to if the time of coagulation falls anywhere between five minutes and nineteen minutes.

THE DETERMINATION OF POTASSIUM IN COLLOIDAL SILVER PREPARATIONS CONTAINING POTASSIUM.

Some colloidal silver preparations contain potassium as an ingredient of the protective colloid in the preparation. The potassium can be determined as follows: Ignite a weighed sample of the preparation at a just barely red heat until completely charred and no more smoke comes off. Treat the ignited residue with hot nitric acid until the silver is dissolved. Dilute with water, filter, add HCl to the filtrate to precipitate the silver as chloride, allow the AgCl to coagulate, filter, evaporate the filtrate to dryness, ignite very gently, cool. Dissolve in water, filter if necessary, add platinic chloride in excess and evaporate to small bulk but not to dryness. Add about 60 Cc. of 80 percent alcohol, allow to stand several hours, filter off the potassium platinic chloride on a Gooch crucible which has been dried at 130° C. and weighed, and wash with 80 percent alcohol. Dry the Gooch and potassium platinic chloride at 130° C. and weigh. The platinic chloride is shown to be in excess by its presence in the filtrate from the potassium platinic chloride. If the platinic chloride was not in excess, the filtrate must be evaporated until free from alcohol, more platinic chloride added and the treatment with 80 percent alcohol repeated.

$$K_2PtCl_6 \times 0.19398 = K_2O.$$

NOTES ON SOME U.S.P. REQUIREMENTS FOR COLCHICINE.

The U.S.P. requires a melting-point of 142.5° C. A fixed temperature like this is impracticable, as slight decomposition occurs around the melting-point. This has been recognized by the U.S.P. Revision Committee and the U.S.P. IX will state that colchicine melts between 142° and 146° C. The melting-point of colchicine is difficult to observe because the melted colchicine does not form a meniscus in the melting-point tube, as occurs with most compounds, but forms globules or wets the inside of the tube. If the point at which a meniscus is formed is taken as the melting-point, a much higher temperature will be obtained as the melting-point. For instance, a sample of colchicine formed globules at 144° C. but did not form a meniscus in the tube until a temperature of 160° C. was obtained.

Most of the colchicine on the market contains chloroform of crystallization. The present U.S.P. does not recognize this fact and offers no requirements regarding it. The U.S.P. IX will require that colchicine lose not more than 5 percent in the heating test. This will prevent excessive chloroform in colchicine. It is probably not practical to prohibit chloroform entirely, as it is exceedingly difficult to completely drive off the chloroform. A sample of colchicine containing chloroform of crystallization which lost 22 percent of its weight when heated for eight hours at 100° C. still contained enough chloroform to give the phenyl-isocyanide reaction. The phenyl-isocyanide reaction, of course, is exceedingly delicate, but

nevertheless this experiment illustrated the difficulty of getting rid of all the chloroform.

A temperature of 100° C. does not appreciably darken the color of colchicine, so that there is no excuse for excessive quantities of chloroform in this product, as simple heating will drive off the chloroform to a satisfactory degree. Colchicine is hydroscopic and the loss on heating requirements of the U.S.P. IX will also prevent excessive moisture in this product. The present U.S.P. hydrochloric acid-ferric chloride-chloroform identification test is unsatisfactory in respect that it requires the mixture to be heated only to boiling. With this procedure a ruby-red chloroform layer may or may not be obtained. If, however, the boiling is continued for one to two minutes, guarding against excessive evaporation, a good reaction is always obtained. With the many samples of colchicine which we have tested, greenish chloroform layers were almost always obtained if the mixtures were only brought to boiling, while if boiled from one to two minutes ruby-red or sometimes brownish tinted ruby-red chloroform layers were obtained.

"CHEEP GAS" TABLETS.

These tablets are sold on the market in Philadelphia for adding to gasoline fuel, with the claim "More mileage—no carbon. One of these tablets to each gallon of gasoline renders combustion complete, keeps cylinders clean, increases the mileage ⅓ to ½." On examination they were found to be grayish, crystalline, mottled tablets with the odor of naphthalene and citronella. Each tablet weighed 8¼ grains and when ground melted at 79½°–80½° C. and boiled at 217° C. As naphthalene melts at 80° C. and boils at 218° C. the tablets are undoubtedly merely naphthalene scented with oil of citronella.

WILFARTH MODIFICATION OF THE KJELDAHL METHOD APPLIED TO PEPTONIZED BEEF, BEEF EXTRACT, AND BEEF, IRON AND WINE FOR ASSAY FOR PROTEIN.

The method adopted is as follows:

Two to three Gms. of the sample (in the case of beef, iron and wine, the residue from 50 Cc.) is placed in the digestion flask, 1 Gm. metallic mercury is added and 25 Cc. of a solution of 200 Gms. of phosphorus pentoxide in 1000 Cc. of concentrated sulphuric acid. After the liquid becomes colorless transfer the acid to the distillation flask with 200–400 Cc. of water. Precipitate the mercury by addition of 25 Cc. of a solution of 40 Gms. of potassium sulphide in 1000 Cc. of water. The acid is neutralized as usual with saturated sodium hydroxide solution. After the addition of a few Gms. of zinc the ammonia is distilled into seminormal acid and the excess of acid is titrated back.

This method is preferable for the determination of beef, iron and wine, beef extract, and peptonized beef, as the time of digestion is reduced from the six or seven hours required by the Gunning-Kjeldahl method to about one or one and one-half hours by the Wilfarth method.

Some comparative results obtained by the two methods are as follows:

	Gunning-Kjeldahl.	Wilfarth.
Sample peptonized beef	62.54 percent	62.10 percent
Sample beef, iron and wine	1.17 percent	1.16 percent
Sample beef extract	45.17 percent	45.10 percent

THE CHEMICAL EXAMINATION OF PYROLIGNEOUS ACID.

The most important constituents of pyroligneous acid, from a commercial stand-point, are acetic acid, acetone, methyl alcohol and phenols. The acetic acid

can be determined by direct titration with standard potassium hydroxide solution, using a large quantity of phenolphthalein test solution as indicator and diluting the sample largely, to overcome the difficulty of observing the end point occasioned by the darkening of the solution when nearing the neutral point.

Acetone can be determined by forming iodoform and shaking it out with ether. The ether is evaporated rapidly and the iodoform weighed immediately. Rather large samples should be used because of the difficulty of handling iodoform without loss by volatilization. The acetone can also be determined by the following method:

Sample 2 Cc., dilute with 40 Cc. water and add 50 Cc. of a mercury reagent made by dissolving 5 Gms. yellow oxide mercury in a warm mixture of 20 Cc. of concentrated H_2SO_4 and 100 Cc. of water. On standing on a water-bath the acetone is precipitated possibly as $2H_2SO_4$. 3HgO. $CO(CH_3)_2$; however, this precipitate is variable and in all cases a check should be run on a facsimile mixture of approximately the same composition. The precipitate is collected on a tared filter and washed with cold water using suction. The filtrate should be treated with more reagent. The precipitate is then dried at 100–110° C. and weighed. From the weight a factor is calculated. This factor is variable according to the composition of the sample, etc. In one sample the factor was 0.052 and in another sample of different composition it was 0.074. The iodoform and mercury methods check up together fairly well. The methyl alcohol can be determined by the following method:

Seven and five-tenths Cc. are treated with potassium carbonate and iodine until the iodoform is completely precipitated. The mixture is made up to definite volume and a filtered aliquot part is placed in a distilling flask. Some sodium thiosulphate and sodium hydroxide is added and the methyl alcohol distilled off. The specific gravity of the distillate is taken at 15.56 percent and compared with the methyl alcohol tables in Van Nostrand's Chemical Annual, 1909. A facsimile preparation of known methyl alcohol content yielded 96.1 percent of its methyl alcohol by this method.

The phenols can be determined by shaking out 100 Cc. with benzol, after adding 5 Cc. H_2SO_4. The benzol solution of phenols is then shaken with a measured volume of 20 percent NaOH solution in a phenol measuring bulb and the increase in the NaOH layer calculated as phenols, 1 Cc. to the gramme.

NOTE ON OPIUM ASSAY.

The impression is quite prevalent that if in an opium assay the morphine is allowed to stand for crystallization for a longer time than the sixteen hours prescribed by the U. S. Pharmacopœia, the morphine will be less pure and more difficult to filter off and wash. In a series of comparative tests of this point the following data were obtained:

Sample.	Time for crystallization.	Insol. in lime water.	Assay.	Remarks.
Gum opium	16 hours	0.094 Gm.	21.03 percent	Not difficult to filter
	44 hours	0.088 Gm.	20.43 percent	Not difficult to filter
Weak solid extract ..	16 hours	0.106 Gm.	6.36 percent	Not difficult to filter
	66 hours	0.128 Gm.	6.96 percent	Not difficult to filter
U. S. Pharmacopœia	18 hours	None	17.80 percent	Not difficult to filter
Solid extract	59 hours	None	18.07 percent	Not difficult to filter
Fluid concentrated	16 hours	None	3.33 percent	Not difficult to filter
	66 hours	None	3.33 percent	Not difficult to filter

It is apparent, therefore, that morphine allowed to stand more than sixteen hours for crystallization is not necessarily less pure or more difficult to wash on the average than morphine which is allowed to stand only the sixteen hours prescribed.

POTASSIUM ARSENITE.

It has often been pointed out that chemicals which are not official and for which no definite authoritative standards have been provided are liable to vary considerably in composition. This point is well illustrated by the potassium arsenite on the market.

Merck's Index gives the formula $KAsO_2$. H_3AsO_3 for potassium arsenite. Until recently we have in our laboratory accepted this formula as the basis on which the strength of potassium arsenite so-called should be based. Recently, however, an American manufacturer has placed on the market a potassium arsenite for which the formula $KAsO_2$ is claimed. Examinations of several lots of this product have shown it to assay 99.2 percent $KAsO_2$ with 0.21 percent water and a trace of carbonate. The importance of making such products official and thereby holding them to a definite standard, even though not used in enormous quantities, is apparent.

NOTE ON THE MEASUREMENT OF UNDISSOLVED ALBUMEN IN THE ASSAY OF PEPSIN.

In the official pepsin assay one-half hour is prescribed for the settling of the undissolved albumen. That this prescribed time is a minimum and not necessarily a maximum is shown by the following experiment:

Experiment number.	One-half hour.	Fifteen hours (over night).
1	50 Cc.	49 Cc.
2	48 Cc.	47 Cc.
3	40 Cc.	40 Cc.
5	37 Cc.	37 Cc.
7	1 Cc.	2 Cc.
9	1 Cc.	1 Cc.
10	Less than 1	Less than 1
XX	Less than 1	Less than 1
XXI	Less than 1	Less than 1

This is of importance to the analyst, as it shows that it is not necessary that the reading of the undissolved albumen should be made exactly one-half hour after the digestion is made. Standing over night apparently yields identical results and thus in necessary cases it allows a pepsin assay to be run in a working time which is too short to allow the undissolved albumen to settle one-half hour.

SAW PALMETTO COUGH PREPARATIONS.

Saw palmetto owes its virtue chiefly to pectoral and sedative activity and therefore finds a place in the treatment of coughs. The active constituents of saw palmetto are oleoresinous in nature and are represented by the oleoresin or alcoholic extract of the drug. Oleoresin saw palmetto can be dispensed in soluble elastic capsules as such, or in combination with terebene, oil sandalwood, creosote, eucalyptol, olive oil, etc. A cordial could be worked out along the lines of the elixir described below.

An elixir of saw palmetto and terpin hydrate can be prepared as follows: Dissolve terpin hydrate 1.75 Gm. in fluidextract saw palmetto 40 Cc. and alcohol 10 Cc.

Add tincture sweet orange peel 1 Cc., solution saccharin 0.2 Cc., glycerin 40 Cc. and syrup 100 Cc. This elixir contains 8 grains terpin hydrate and 184 grains saw palmetto per fluidounce. This elixir may find its use in supplying the apparent need for a terpin hydrate elixir with sedative properties and without "dope." Saw palmetto oleoresin or alcohol-extractive, which is a clear green oily liquid, could be included as an ingredient of a cod liver emulsion, although the green color might be objectionable. The alcohol-extractive of saw palmetto is apparently soluble enough in glycerin to admit of the preparation of a glycerite either by percolation or maceration or by saturating glycerin with the alcohol-extractive of the drug.

Saw palmetto is probably not adaptable to tablets, lozenges or pastilles, as it is oily and has a large dosage.

A clear simple syrup of saw palmetto can be prepared as follows:

Saw palmetto 15 Gms. (or less), exhaust with alcohol. Recover alcohol. Add 15 Cc. glycerin, warm and stir for a few hours. Add 70 Gms. sugar and then water q.s. 100 Cc., warming a little to dissolve sugar.

A complex syrup can be prepared by incorporating the alcohol-extractive from 15 grains saw palmetto in 1 fluidounce of syrup white pine compound.

WOOL-FAT SUBSTITUTES.

In nearly all the European pharmaceutical papers, especially those of Sweden, Switzerland, and Holland, recipes are published for substances which can be used in the place of wool-fat, which is very scarce in all the countries that were in the habit of obtaining it from Germany. The stocks in Germany itself appear to be exhausted now. The "Pharm. Zeitung" recommends as a wool-fat substitute a mixture of 3 percent of stearinic anilide and 97 percent of vaseline (fetron). It is also proposed to make use of Chinese wood-oil for this purpose. According to a German patent Chinese wood-oil can be converted into a solid fat by heating it to 300° C. for a short time. Three parts of this solid fat dissolved in 7 parts of ordinary liquid Chinese wood-oil by warming and then adding 3 parts of wax gives a product which is said to have exactly the same physical properties as lanoline. Professor P. van der Wielen, in dealing with the matter in the "Pharmaceutisch Weekblad," confesses that there is no really good substitute for wool-fat. Of many combinations that have been tried, he recommends as one of the best substitutes a mixture of 20 parts of white beeswax and 80 parts of fresh linseed oil, which absorbs about 170 parts of water. Other mixtures which answer the purpose fairly well are the folowing: (1) Linseed oil, 20 parts; white vaseline, 20 parts; and spermaceti, 5 parts. This absorbs about 100 percent of water. (2) Yellow beeswax, 10 parts; wool-fat, 25 parts; vaseline, 45 parts; water, 25 parts. (3) Yellow beeswax, 15 parts; yellow vaseline, 60 parts; and water, 25 parts. The Swedish pharmaceutical paper, "Svenk. Farm. Tidskr.," publishes a recipe for "Cenolinum anhydricum," which consists of white vaseline 90 parts, cetyl alcohol 3 to 5 parts, and wool-fat 10 parts. Cenolinum is a mixture of equal parts of cenolinum anhydricum and water. According to Professorl van der Wielen a substitute of the same properties is obtained without the cetyl alcohol. Another recommendation is for a substance called "Cerolanum anhydricum," consisting of yellow beeswax 7 parts, wool-fat 15 parts, and white American vaseline 78 parts. Cerolanum is a mixture of 70 parts of cerolanum anhydricum and 30 parts of water. In the "Schweiz. Apoth. Zeitung" a correspondent prefers a mixture of yellow beeswax 3 parts, solid paraffin 7 parts, and yellow vaseline 90 parts.—*Chemist and Druggist.*