ON THE ESTIMATION OF TANNINS, WITH CRITICAL NOTES ON THE METHODS OF HAMMER AND LOEWENTHAL.

By Henry M. Rau.

In connection with the manufacture of extracts from sumac and chestnut wood, I have had to make frequent estimations of the tannin in the raw materials—leaves, wood, etc.—and in the liquors obtained at various stages of the manufacture. I had likewise to make such estimations in mixtures of sumac and chestnut wood extracts with other extracts employed as dyestuffs.

Hammer's and the most recent form of Loewenthal's methods were employed, and a large number—not far from fifty—estimations of tannin in various materials were made by each of the methods. Special precautions were taken to reduce all errors of execution to a minimum, as will be explained in detail.

While the results obtained in repeated analyses of the same material by one method or the other agreed fairly well when the conditions of the analysis were not varied, it was found that apparently the most trivial modifications of these conditions occasioned quite discordant results. Furthermore, it became evident that if the results in certain cases were correct, those in others—the conditions not being varied—were obviously much too high. In general, this was found when the materials contained any considerable proportion of gallic acid or coloring matters.

It is well known that decoctions of sumac are liable to a spontaneous fermentation, by which the tannin is converted into gallic acid. In several instances, in which this conversion was very marked, both Hammer's and Loewenthal's methods returned almost as great a percentage of tannin as was found in the same materials before any fermentation had taken place. Again, in estimations made with mixtures of logwood extract, containing but very little tannin, and sumac extract, the results were more than twice as high as they should have been.

Proctor and others have called attention to the error of Hammer's method, produced by the presence of gallic acid in the material of analysis. To the best of my knowledge, however, the extent of this

error has not been adequately determined, nor has it been shown that the presence of coloring matters also seriously effects this and Loewenthal's methods. Before proceeding to the investigations which I made in this direction, certain definitions and ascertained facts in regard to the nature of "tannins" deserve a place.

The term "tannin" designates not a distinct substance but a class of vegetable principals—most of them unknown in the pure state—which possess an astringent taste, precipitate solutions of gelatin, albumen, certain alkaloids and metallic bases, and which combine in a peculiar manner with the gelatine producing animal tissues—as with the corium of the skin to produce leather. Notwithstanding the views to the contrary of Darton*, all the evidence at present possessed points to the existence of a number of tannins, which, while they have the above described properties in common, differ in composition and in the derivatives which they yield. Investigations of oak and hemlock tannins have been made by Oser,† Böttinger,‡ and Etti,‖ which prove that these tannins are distinct one from the other as well as from the best known of the tannins, that of nutgalls. This last, commonly called tannin or tannic acid, but more specifically to be designated gallotannic acid, is the only body of the class of which our knowledge is, in a measure, definite. Originally prepared from nutgalls, it has been identified, chiefly through Stenhouse's researches, in a few other materials, such as sumac and divi-divi.

The distinguishing property of this tannin is its convertibility into gallic acid by the action of certain ferments and by heating with mineral acids and alkalies. Gallotannic acid has also been obtained by synthesis from gallic acid. Similar treatment of the tannins of oak and hemlock barks, chestnut wood, etc., fails to produce gallic acid; on the contrary, entirely distinct derivatives are obtained. Inasmuch as these distinctions are disregarded by all the methods of tannin estimation, owing to the imperfect knowledge which we possess of the majority of the tannins, it follows that tannin materials of one variety cannot, in strictness, be analytically compared with those of another.

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* Journ. Amer. Chem. Soc., IV., 49.

† Sitz. ber. d. math. naturio., Klasse. d. Akad. in Wien, 72., 186.

‡ Berichte XIV., 1599, 2390; XVI., 2710; XVII., 1041, 1123.

‖ Berichte XIV., 998, 1826; XVI., 2304; XVII., 1820.

The statement, for example, that sumac contains twice as much tannin as oak bark, cannot be considered scientifically accurate.

Passing over this point, it is next to be determined what conditions must be fulfilled by any method by which estimations of tannin in materials of the same variety give results reliable for comparison. A quantitative analysis divides itself into; the separation of the substance to be determined from accompanying substances, as far as the latter may affect the result, and the quantitative estimation proper. The latter may be gravimetric, volumetric, etc. In nature the tannins do not occur in the pure state but are accompanied by, and in some instances combined with, certain substances of the sugar group, with pectine, coloring matters and gallic and ellagic acids, besides other ordinary constituents of vegetable organisms. In the arts, moreover, extracts containing tannins are often employed in mixture with such dyestuffs as logwood and redwood extracts, in which, as they are generally employed for purposes of adulteration, they have occasionally to be determined. A separation from its accompanying impurities is, therefore, essential to every method of estimating tannin, and on the correct and perfect separation the value of the method depends in great part. Classified on this principle, the best known methods are based:

1. On precipitation of the tannin with gelatin or isinglass solution: Davy's, Fehling's, Hallwach's, Lipowitz's, F. Schulze's and one form of Loewenthal's method.

With the exception of the last, these methods have not remained in general use; and I do not propose to discuss them, further than to state that they are cumbersome and exposed to many sources of error.

2. On precipitation of the tannin with the sulphate of cinchonine solution: Wagner's.

3. On precipitation of the tannin with solutions of certain metallic salts and bases: Fleck's, with cupric acetate. Pribram's, with plumbic acetate. Persoz's, with stannous chloride. Gerland's, with tartar emetic. Grassi's, with barium hydrate.

These methods are unquestionably very faulty, inasmuch as with the tannin are precipitated not only gallic acid and coloring matters, but various other substances contained in vegetable extracts. Villon * has recently proposed a modification of Pribram's process,

* Bul. Soc. Chim., 47, 97.

stating that tannin but not gallic acid or analogous substances are precipitated by plumbic acetate. This I am constrained to deny on the basis of careful experiments, which I made both with gallic acid and various coloring matters.

4. On absorption of the tannin by gelatine producing tissue: Hammer's, Loewenthal's (with in its recent modification.)

These methods have obtained the most general recognition. The former has been accepted as the standard by which all the more recently devised methods have been gauged, while Loewenthal's has a quasi official stamp through its adoption by some of the principal associations connected with the tanning industry in Europe.

Hammer's method has been developed in two directions, a gravimetic and a specific gravity form being in use.

Loewenthal's original method underwent repeated alterations. In the form in which it belongs in this group, it is practically a volumetric modification of Hammer's. The essential conditions which both methods must fulfill, if accurate results are to be expected; may be stated thus: absorption of the tannin by gelatine producing tissue must be complete, and no other substances (non-tannins) must be removed or affected thereby.

If the accordance of the methods with the first of these conditions is investigated, it is found that, proper precautions being adopted, no errors can be due to this source.

50 c.c. of a 3 per cent. solution of gallotannic acid (*tanninum puriss.*) were treated for twenty-four hours with 6 grammes of the bone tissue described by Simand,* previously moistened with water. The solution was afterwards filtered and evaporated to about one-fifth its original volume, and tested with gelatine solution. As not the slightest precipitation was produced, it proved that the tannin had been completely absorbed. The result was the same with decoctions of sumac, oak bark and chestnut wood, as also when in place of the bone tissue, Hammer's ** skin powder was employed.

It is otherwise when the second of the conditions is applied, and I shall show that the erroneous results often obtained are in the case of Hammer's method wholly and in that of Loewenthal in part, traceable to the absorption of non-tannins by gelatine producing tissue.

* Fresenius' Quant. Anal., II., 621.
** Fresenius' Quant. Anal., II., 626.

Critical Notes on Hammer's Method.*

In the experiments to be detailed in the following, the specific gravity form of Hammer's method was the subject of special examination, but the principal facts adduced apply equally to the gravimetric form. According to Hammer, a solution or decoction of the material of analysis is prepared in known proportions of substance and solvent (distilled water). If the material contains pectine, this has to be removed according to the process suggested by Lowe.† A portion of the solution is digested with specially prepared gelatine producing tissue—Hammer used skin powder—in such wise that the original concentration is unaltered or altered in a known ratio. After sufficient digestion with the skin powder to ensure complete absorbtion of the tannin, the solution is filtered, and its specific gravity and that of the original solution are determined. The difference in specific gravity corresponds to a certain percentage of tannin, and from this percentage the amount contained in the material of analysis is calculated. The "error of observation" which enters into determinations of this kind becomes relatively smaller, as the difference in specific gravity becomes greater; for which reason it is advisable not to take the original solution too dilute, and if possible to use such proportions of substance and water that the solution may contain from 3–5 per cent. tannin. In connection with his method Hammer prepared a table giving the percentage of gallotannic acid corresponding to densities from 1.0000–1.0201 of its solutions in water. Strictly considered calculations based on this table do not hold good for tannins other than gallotannic acid, but the probable error from this cause is so slight that it may be neglected. On the other hand it is to be noted that Hammer prepared his table by determination of the density of pure aqueous solutions of gallotannic acid, and that it is probable that the soluble principles which are present with the tannins, have some influence on the specific gravity which the table does not take into account. That this objection is not without some foundation, is evidenced by the following experiment:

A 5 per cent. solution of gallotannic acid (*tanninum puriss.*) had sp. gr. 1.01841. The density of distilled water being 1.0000,

* Journ. f. Prakt. Chem., 81, 159.
† Zeit. f. anal. Chem., 4, 368.

the 5 per cent. gallotannic was represented by the increase in sp. gr. 0.01841. A solution of cane sugar was prepared having sp. gr. 1.01646, and 5 per cent. of the same gallotannic acid dissolved therein. The sp. gr. of the latter solution was then found to be 1.03398. Consequently the same percentage of tannin was in this case represented by a somewhat smaller increase in sp. gr., 0.01752.

The errors which, in practice, arise from similar causes, are generally much smaller, and, as their exact extent can hardly be determined, must be overlooked. Hammer has stated that for the absorption of the tannin, a quantity of skin powder about four times the weight of tannin contained in solution were sufficient. He also considered a very brief digestion, say of fifteen minutes, sufficient to absorb the whole of the tannin.

Later it was found that so short a time did not always suffice, and to be quite safe a digestion of about twenty-four hours was found advisable. Hammer's skin powder is advantageously replaced by Simand's bone tissue, as the latter is generally obtainable, readily purified and after purification yields to water less soluble substance than skin powder. Simand's statement to this effect was confirmed by my own experiments.

10 grms. carefully prepared bone tissue were twice digested, each time for twenty-four hours, with 100 c.c. distilled water at a temperature of about 15°. The united filtrates, evaporated and dried at 100°, left a residue weighing 0.0245 grms., too small a quantity to have any appreciable effect in determinations by the specific gravity method. Although bearing on Loewenthal's rather than on Hammer's method, I may state in this connection that the soluble substance of bone tissue exerted a scarcely perceptible effect on permanganate solution.

For making the sp. gr. determinations I employed a very delicate Geissler's pyknometer with capillary neck and glass cap ground on to prevent evaporation during the weighings, and provided also with a thermometer divided into $\frac{1}{10}°$.

To calibrate the pyknometer, it was weighed a number of times filled with water at various temperatures, and from the weight of the water contained at these temperatures the mean weight for water of 0° was calculated. From this a table was prepared showing the calculated weight of distilled water contained at temperatures from 15°–25°. By employing the table a single weighing

and observation of temperature sufficed to determine the sp. gr. of the solutions subsequently tested. For the sake of greater accuracy, however, three determinations at different temperatures were made in every instance. All the weighings were reduced to vacuo, but I am satisfied that this was an unnecessary refinement. For absorption of the tannin, the required amount of bone tissue was weighed off in a small, dry flask, and moistened with exactly 50 c.c. distilled water, measured from a Geissler burette. The flask was allowed to stand for one hour so that the water might thoroughly swell the bone tissue. 50 c.c. of the tannin solution were then added in the same manner.

The contents of the flask were then allowed to digest for twenty-four hours, shaking the same at intervals, and were afterwards filtered into a dry flask. The filtrate was then ready for the sp. gr. determination. In every case a small portion was concentrated by evaporation and tested with gelatine solution to make sure that the tannin had been completely absorbed.

On the other hand, the remainder of the original solution was diluted with exactly its own volume of water so as to present the same concentration as the portion treated with the bone tissue.

In giving the sp. gr. as found I have designated the original solution, thus diluted, by the letter A, and that portion treated with bone tissue by the letters X, Y or Z.

As the manipulation was the same in every case, I will not repeat it in detail with each of the following experiments :

ABSORPTION OF GALLIC ACID BY GELATINE-PRODUCING TISSUE.

The gallic acid was purified by repeated crystallization from hot water, and dried in vacuo over sulphuric acid ; a determination of water of crystallization was made.

0.9075 grms. dried at 105° lost 0.0869 moisture = 9.57 per cent.
The formula $C_7H_6O_5 + H_2O$ requires moisture = 9.55 per cent.

FIRST EXPERIMENT.

1.4540 grms. gallic acid were dissolved in 181.75 c.c. distilled water, 50 c.c. were treated with 3 grms. bone tissue and 50 c.c. water [X]. The remainder was diluted with exactly its own volume of water [A].

A had the sp. gr.	1.00151	1.00151	1.00153	Average 1.00152
X " " "	1.00129	1.00128	1.00131	" 1.00129

The decrease in sp. gr. is evidence that a certain amount of gallic acid was absorbed, and as for solutions as dilute as these, it may be assumed that the excess of sp. gr. over that of water, 1.00000 is proportionate to the percentage of dissolved substance, it follows that nearly 16 per cent. of the gallic acid in 50 c.c. of the solution were absorbed by the 3 grammes of bone tissue. Whether or not the absorption of gallic acid depends on a chemical combination with the tissue, as is the case with tannin, must be left undecided.

Washing with cold water did not remove the gallic acid which had been taken up by the bone tissue.

Second Experiment.

2.0000 grms. gallic acid were dissolved in 250 c.c. distilled water. The solution therefore had the same concentration as in the previous experiment. The conditions were all in respects as before, except that double the quantity, 6 grms., of bone tissue were used.

A had the sp. gr. ....1.00151 1.00150 1.00151 Average 1.00151
X " " " ....1.00110 1.00112 1.00112 " 1.00111

The decrease in sp. gr. in this case was equivalent to an absorption of 27 per cent. of the gallic acid. Hence gelatine-producing tissue not only absorbs gallic acid from aqueous solution, but the amount absorbed varies with the quantity of the tissue, and probably with the concentration of the solution and duration of the digestion also. The facts adduced in these experiments are to be borne in mind in connection with the three following :

In the third and fourth experiments so called c. p. tannic acid (Schuchardt's) was used ; in the fifth a mixture of the same with gallic acid. The so called c. p. tannic acid is by no means pure gallotannic acid ; in fact, an absolutely pure article is something that I have not been able to obtain, nor yet to prepare by any of the methods described in the text books. I must also express doubt whether those who, before me, have treated of this subject, employed pure gallotannic acid in their experiments. The article which I used contained, besides moisture, about 7 per cent. of impurities, as will appear in the following : Of these impurities, gallic acid formed the greatest part, as was ascertained by dissolving some 10 grammes in water, extracting the tannin with bone tissue, and concentrating

the detannized filtrate, which yielded a crystalline residue showing all the reactions of gallic acid. It has been stated that pure gallotannic acid is not hygroscopic. The article which I used, however, showed slight increases in the percentage of moisture, when tested from time to time.

Third Experiment.

The c. p. tannic acid contained 89.3 per cent. dry residue (dried at 105°) 3.7025 grms. (3.3063 dry) were dissolved in 123.4 c.c. distilled water; 50 c.c. were treated with 6 grms. of bone tissue and 50 c.c. water [X]. The remainder was diluted with exactly its own volume of water [A].

A had the sp. gr.....1.00539 1.00545 1.00547 Average, 1.00544
X " " " ....1.00053 1.00053 1.00052 " 1.00053

Decrease in sp. gr.=0.00491 which corresponds by Hammer's table to 1.23 per cent. tannin in A. The original solution was made up of 123.4 grms. water and 3.7 grms. substance=127.1 grms. This diluted with an equal volume of water weighing 123.4 grms. gives 250.5 grms. If 250.5 grms. contained 1.23 per cent. tannin, the total amount of the latter=$\frac{250.5 \times 1.23}{100}$=3.0811 grms. This being the amount contained in 3.3063 grms. of c. p. tannic acid, dried at 105°, the latter was 93.2 per cent. strong.

Fourth Experiment.

The same c. p. tannic acid was used; it had attracted a little moisture and contained *89.0* per cent. dry residue.

5.0430 grms. (4.4883 dry) were dissolved in 150 c.c. distilled water, giving a solution slightly more concentrated than in the previous case.

The conditions of the test were as before, except that a larger quantity—9 grms.—of bone tissue were used.

A had the sp. gr.....1.00604 1.00606 1.00607 Average, 1.00606
X " " " ....1.00051 1.00054 1.00047 " 1.00050

Decrease in sp. gr.=0.00556 corresponding by Hammer's table to 1.39 per cent. tannin in A. Making the calculation as before the total amount of tannin=4.2395 gr. As this amount was contained in 4.4883 grms. c. p. tannic acid dried at 105°, the latter was 94.4 per cent. strong. The result is higher by 1.2 per cent. than in the preceding experiment, and as the substance was the same in

both cases, the higher result must have been due to the use of the larger quantity of bone tissue. A greater proportion of the gallic acid present as impurity was absorbed and estimated as tannin. Hence it is probable that both results were too high, but for the purpose of the next experiment the average, *i. e.*, 93.8 per cent., was assumed as correct.

Fifth Experiment.

There were taken 3.6787 grms. c. p. tannic acid=3.2851 dry and containing (as calculated from the average of the 3d and 4th experiments) 3.0814 grms. pure tannin, and 1.2147 grms. pure gallic acid. Together they were dissolved in 175 c.c. distilled water. 50 c.c. were treated with 8 grms. of bone tissue and 50 c.c. water [X]. The remainder was diluted with exactly its own volume of water [A].

A had the sp. gr....	1.00493	1.00492	1.00493	Average, 1.00493
X " " "	1.00123	1.00116	1.00118	" 1.00119

Decrease in sp. gr.=0.00374, which corresponds by Hammer' stable to 0.94 per cent. tannin in A. Making the calculation as before the total amount of pure tannin=3.3361 grms. But, as but 3.0814 grms. pure tannin (calculated) had been taken, there was an apparent result=108.2 per cent. It follows that in a mixture containing less than one-third gallic acid, the error due to the absorption of the latter by bone tissue amounted to over 8 per cent. Hence it appears that in the presence of any appreciable amount of gallic acid, estimations of tannin by Hammer's method are apt to be more or less erroneous.

On the Absorption of Coloring Matters by Gelatine-Producing Tissue.

In making the experiments in this direction it would have been preferable to use such coloring matters as accompany the various tannins in nature—yellow coloring matter of sumac, red coloring matter of hemlock and oak bark—but as the preparation of these in the pure state offers very great difficulties, certain dyestuffs were employed instead, which in their general character approximate to those which accompany the tannins. By analogy, the results obtained with the former may be applied to the latter.

Sixth Experiment.

A pure fluid extract of logwood was diluted with cold water, the solution filtered and evaporated to dryness. To remove a small quantity of pectine, the residue was taken up in 75 per cent. alcohol, the alcoholic solution filtered, evaporated and the dry residue again dissolved in cold water. Thus prepared, the solution was of a deep reddish yellow color, had no perceptibly astringent taste, and contained essentially the coloring principles of logwood—hematoxyline and hemateine—only.

50 c.c. were treated with 10 grms. of bone tissue and 50 c.c. water [X]. The remainder was diluted with exactly its own volume of water [A].

A had the sp. gr....	1.00570	1.00568	1.00571	Average, 1.00570
X " " "	1.00328	1.00330	1.00335	" 1.00331

The great decrease in sp. gr.=0.00239 was obviously not due to the absorption of tannin, of which at most the logwood could contain but a trace. It was therefore due to the absorption of the coloring matters, a result confirmed by treating the residual bone tissue—after washing with water—with a weak solution of potassic dichromate, when the bone tissue assumed the deep blue black color characteristic of the logwood principles in the same reaction.

The deep red yellow color of A was reduced in X to a light straw yellow. A second treatment of X with bone tissue reduced its sp. gr. to 1.00224.

Seventh Experiment.

The material in this experiment was extract of Persian berries, a yellow dyestuff, to which the yellow coloring matter of sumac shows much similarity. The results were the same as with logwood extract.

Reasoning from analogy, it may be concluded that the coloring matters which are never absent in tanning materials would in like manner be absorbed by gelatine producing tissue, and that in such cases also estimations of tannin by Hammer's method are certain to be erroneous.

CRITICAL NOTES ON LOEWENTHAL'S METHOD.*

In advance of Loewenthal, Monier** employed potassium permanganate for the titration of tannin. He simply added the standard solution of permanganate until, by a slight excess thereof, a permanent reddish tinge appeared. Loewenthal found that when tannin was titrated with potassium permanganate in acidulated solution and in the presence of a sufficient excess of indigo carmine, the oxidation proceeded in such wise, that with the complete decolorization of the indigo—indicated by the solution assuming a pure yellow color—the tannin was completely destroyed. At first Lowenthal supposed that the substances which accompany the tannin were not affected by permanganate; this was disproved by Neubauer† who suggested pure animal charcoal as a means of removing the non-tannins, supposing that the tannin alone was absorbed by animal charcoal.

This separation was found to be quite erroneous, and then Loewenthal†† himself amended his original process by employing a solution of gelatine saturated with salt for the precipitation of the tannin. In this form the method is still extensively in use, and belongs in the first group of methods referred to in this paper. It is therefore open to the same objections as all the methods which depend on the precipitation with gelatine. These objections are, in brief, that gelatine precipitates certain coloring principles, and that the precipitate of tannate of gelatine is to some extent soluble in an excess of the precipitant. Siemand§ therefore proposed the absorption of the tannin, as in Hammer's method, by gelatine producing tissue of skin, bone or horn. Hence my characterization of this form of Loewenthal's method, which I believe to be the one most generally used, as a volumetric modification of Hammer's.

It would, therefore, require only that the solution containing tannin be titrated in connection with a sufficient quantity of indigo carmine, and again with the same quantity of the latter after the removal of the tannin, to show by the difference the amount of permanganate required by the tannin itself. If, furthermore, the

* Jourr. f. Prakt. Chemie, 1860, **3**, 150.
** Comptes rendus, **46**, 44.
† Zeit. f. anal. Chem., **10**, 1.
†† Zeit. f. anal. Chem., **16**, 33, 201, **20**, 91.
§ Zeit. f. anal. Chem., **22**, 595.

permanganate solution has been standardized by means of a tannin of known strength, a simple calculation would give the amount of tannin in the solution and thus in the material of analysis.

As the standard, Neubauer adopted gallotannic acid, whose degree of purity he established by Hammer's method. But as pure gallotannic acid is scarcely obtainable, he determined the ratio which gallotannic and oxalic acids bear to each other in respect of their oxidation by permanganate (oxidation ratio) so as to be able to standardize solutions of the latter by means of pure oxalic acid, which is easily prepared. He found that 63 parts of oxalic and 41.57 parts of gallotannic acid were equivalent. These figures have been drawn in question by Councler and v. Schroeder,* who stated the ratio as 63:34.25. On the other hand, Neubauer's results have been confirmed by Oser** and Ulbricht.† As all these observers are known as experienced and careful analysts, it is reasonable to ascribe such remarkable variations to some defect in the method by which their results were obtained. The fact is that there are more than a single defect to be noted. In the first place, these chemists, in determining the ratio between gallotannic and oxalic acids, used a more or less impure gallotannic acid, *i. e.*, one containing more or less gallic acid. For determining its degree of purity they relied on Hammer's method, and this, as has been shown, yields, in such cases, results that may vary considerably. My own experiments made to determine this ratio, if possible, with greater exactness, were made with the c. p. tannic acid already referred to. My results agreed much better with those of Councler and v. Schroeder than with those of Neubauer.

There is, however, another more important defect peculiar to the Loewenthal method itself. This is alluded, but without any attempt at explanation, by Ulbricht.†† He indicates that not alone the concentration of the solution and the quantity of indigo carmine, but such details as the time and manner in which the permanganate solution is added exert a very great influence on the result of the titration. Repeated experiments of my own have abundantly confirmed the force of these observations. Ulbricht's conclusion is that in order to make estimations of tannin by Loewen-

* Berichte XV., 1373.

** Sitzungsber d. math. naturlo, Klasse der Akad. in Wien, 72, 186.

† Annalen d. Oenologie, 3, 63.

†† Berichte XVIII., 1116.

thal's method comparable one with another, a uniform manipulation extending to the slightest details should be adopted by all chemists. My conclusion is that these details of manipulation exert so variable an effect, that Loewenthal's method becomes exceedingly cumbersome, and, in the end, of very doubtful value.

In the following it is proposed to point out why these apparently trivial differences in the manner of titration exert such untoward effects, and practically render the method uncontrollable.

The details observed in my investigations of Lowenthal's method were:

1. Preparation of the permanganate solution. One gramme dry crystallized potassium permanganate was dissolved in one litre of distilled water. Special care was taken to exclude from the solution dust or organic matter, but as, nevertheless, it was apt to vary slightly in strength by long standing, its titre was determined anew each day during the course of the experiments, by means of pure oxalic acid.

The latter was prepared pure by crystallization first from absolute alcohol and then from water, and dried in vacuo.

2. Preparation of the indigo solution. As the best of commercial indigo carmine contains ingredients which render the end reaction—striking of a pure yellow color—indefinite, I prepared a solution of crystallized pure indigotine instead. One litre of this contained about 3 grammes of indigotine and 60 c.c. of conc. sulphuric acid. It was filled into small vials, which, after heating in the waterbath to 70°, were securely sealed and preserved for future use. The solution did not perceptibly change in strength in the course of six weeks.

3. The Geissler burettes employed in the titrations were divided into $\frac{1}{10}$ cc., and even $\frac{1}{20}$ cc. could be noted without difficulty.

They were carefully calibrated and adjusted one to another. The beakers in which the solutions were titrated were selected as nearly as possible of the same shape and dimensions.

The individual features of the method were then investigated in the following order: the titration of the indigo solution, the titration of tannin with the indigo solution, the absorption of the tannin and titration of the non-tannins with indigo solution, establishment of the titre of the permanganate solution by oxalic acid, the ratio between gallotannic and oxalic acid, and the application of this ratio in the case of other tannins.

Titration of the Indigo Solution.

The same quantity—20 c.c. or 40 c.c—of indigo solution was mixed with $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{1}$ litre distilled water; in each of these concentrations repeated titrations were made. The beaker was placed in a white porcelain dish and the liquid continuously stirred during the addition of the permanganate. In some cases the latter was added rapidly, in others slowly, but this had no effect on the result. The end reaction was the disappearance of the last greenish tinge and the taking on of a pure yellow color by the liquid. As the power of the eye to distinguish slight differences of shade is not the same with each person, the so called "personal error" is not without effect. This error disappears when all the observations are made by one person.

The degree of concentration, on the other hand, exerts an appreciable effect, as shown by the following titrations:

40 c.c. indigo solution in $\frac{1}{1}$ litre water . required 36.9 c.c. permanganate

40 c.c.	"	$\frac{1}{1}$	"	"	"	36.85	c.c.	"
40 c.c.	"	$\frac{1}{1}$	"	"	"	36.8	c.c.	"
40 c.c.	"	$\frac{1}{1}$	"	"	"	36.8	c.c.	"
40 c.c.	"	$\frac{1}{2}$	"	"	"	37.6	c.c.	"
40 c.c.	"	$\frac{1}{2}$	"	"	"	37.6	c.c.	"
20 c.c.	"	$\frac{1}{1}$	"	"	"	18.25	c.c.	"
20 c.c.	"	$\frac{1}{1}$	"	"	"	18.2	c.c.	"
20 c.c.	"	$\frac{1}{1}$	"	"	"	18.3	c.c.	"
20 c.c.	"	$\frac{1}{2}$	"	"	"	18.45	c.c.	"
20 c.c.	"	$\frac{1}{2}$	"	"	"	18.4	c.c.	"
20 c.c.	"	$\frac{1}{2}$	"	"	"	18.4	c.c.	"
20 c.c.	"	$\frac{1}{2}$	"	"	"	18.35	c.c.	"
20 c.c.	"	$\frac{1}{4}$	"	"	"	18.7	c.c.	"
20 c.c.	"	$\frac{1}{4}$	"	"	"	18.8	c.c.	"

When the same concentration was maintained, the titrations were very exact, agreeing for the most part within $\frac{1}{10}$ c.c. It required but a single drop of the permanganate to produce the change from a decided greenish yellow to the pure yellow tint. I found the concentation 20 c.c. indigo solution in $\frac{1}{2}$ litre water most convenient, and adopted it in the succeeding titrations.

TITRATION OF TANNIN TOGETHER WITH INDIGO SOLUTION.

A solution was prepared containing in the litre about 2 grammes of the c. p. tannic acid employed in the previous experiments with Hammer's method. In accordance with Neubauer's directions, the quantity of tannin solution taken for the titration was such that it required not much over half as much permanganate as the 20 c.c. of indigo solution. The permanganate was added drop by drop, while the liquid in the beaker was stirred continuously. The flow from the burette was so regulated that the addition of the permanganate was effected in not less than three nor more than five minutes. This point, as will be seen, exercises a considerable influence on the result. A great number of titrations carefully conducted, proved that when tannin and indigo solution were titrated together, the end reaction is not sharp, as is the case with indigo solution alone. Determinations made within the same hour varied as much as $\frac{4}{10}$ c.c. It generally required from 8 to 10 drops of permanganate to produce the change of color from the faintest greenish yellow to the pure yellow, whereas with indigo solution alone, this was effected by a single drop. Variations no greater than these bring about very considerable differences in the calculation of the result. While all the other details of the titration remained absolutely the same, it was observed that less permanganate was required when the drops followed each other slowly—say thirty per minute—than when the addition of permanganate was more rapid—say one hundred per minute. But even when there was no noticeable difference in the rate of adding the permanganate, the results were not uniform.

What may be called "inertia" seemed to retard the oxidation of the tannin in some cases more than in others, and it is to this peculiar feature that I ascribe the widely varying results of Neubauer, Councler and v. Schroeder and myself in determining the "oxidation ratio" between gallotannic acid and oxalic acid. When this peculiar and disturbing feature was further investigated, it led to the following conclusion:

The premise on which Loewenthal's method is founded, viz.: "that with the complete decolorizing of the indigo the tannin has been completely oxidized" is not correct. The fact is, that for the perfect oxidation of the tannin a much greater quantity of permanganate is needed than shown by Loewenthal's method;

when the pure yellow color indicates the complete oxidation of the indigo, the tannin has not been oxidized completely, but to some intermediate stage only. This is made certain not only by theoretical considerations, but by a number of experiments, in which gallotannic acid was titrated with permanganate, first, with indigo solution as indicator, and again without it by Monier's method. A gallotannic acid containing about 2 grammes of Schuchardt's c. p. tannic acid per litre was prepared. Titrated by Loewenthal's method

20 c.c. indigo solution + 5 c.c. tannin solution

				required	26.9	c.c. permanganate.
"	"	"	"	"	27.1	c.c. "
Average					27.0	c.c. "
20 c.c. indigo solution required					18.35	c.c. "
therefore, 5 c.c. tannin solution required					8.65	c.c. "

On the other hand, 5 c.c. solution were added to 125 c.c. distilled water and acidulated with the same quantity of sulphuric acid as was contained in 20 c.c. of indigo solution. The liquid was rapidly heated to about 60° and titrated with the permanganate. Up to a certain point—15 c.c. or 16 c.c. having then been added—the disappearance of the permanganate was quite rapid, but gradually became more tardy until, towards the end, it took from one to two minutes for the disappearance of the drops. The liquid became slightly turbid, as it had also done in the titration by Loewenthal's method. When, after a space of fifteen minutes, the coloration produced by the last drop of permanganate was found to be permanent, 26.4 c.c. had been used. A second titration was made in the same manner, and gave 26.8 c.c. In order to reach the result in a modified manner, 5 c.c. of the solution were added to 125 c.c. distilled water and acidulated with a somewhat greater quantity of sulphuric acid. An excess of permanganate (50 c.c.) was quickly added, producing a brownish red turbid liquid. This was heated, as rapidly as possible, to about 60°, and the excess of permanganate determined by an oxalic acid solution, of which 1 c.c. required exactly 1 c.c. of the permanganate. On the addition of the oxalic acid the liquid became gradually decolorized and finally colorless. When this point was reached, 23.7 c.c. of the oxalic acid solution had been used. The excess of

permanganate was 23.7 c.c., leaving 50 c.c. – 23.7 c.c. = 26.3 c.c. as the amount required by 5 c.c. of the gallotannic acid solution. The results of three determinations, in which the tannin was directly titrated with permanganate, were 26.8 c.c., 26.4 c.c., 26.3 c.c., an average of 26.5 c.c. When the same quantity of tannin was titrated with indigo solution as an indicator, only 8.65 c.c. permanganate were used. Under the conditions of Loewenthal's test, complete oxidation of the tannin is consequently not attained. In Crace-Calvert's "Dyeing and Calico Printing," ed. Stenhouse & Groves, p. 315, the statement is made that gallic acid is oxidized by permanganate into carbonic acid and water, and that Morin (Monier?) based hereon a method for estimating gallic acid. But it is certain that in cold, dilute solutions, such complete oxidation does not take place; for no evolution of carbonic acid can be detected under these conditions.

Absorption of the tannin and titration of the non-tannins. Simand's form of Loewenthal's method employs, like Hammer's, gelatine-producing tissue for the separation of tannin from other ingredients. The solutions operated on are more diluted than in Hammer's method, but, on the other hand, a much greater proportionate quantity of skin powder or bone tissue is used as a rule. The error caused by absorption of non-tannins by these materials becomes relatively greater under these circumstances. This is evidenced by the following experimental results:

First Experiment.

A solution containing 2 grms. of pure gallic acid per litre was prepared, 50 c.c. were treated with 5 grms. bone tissue and 50 c.c water [X]. Another portion was diluted with exactly its own volume of water [A].

20 c.c. indigo solution + 10 c.c solution A	required	29.65 c.c. permanganate.
" " " "	29.7 c.c.	"
Average	29.68 c.c.	"
20 c.c. indigo solution required	18.4 c.c.	"
therefore 10 c.c. solution A required	11.28 c.c.	"

20 c.c. indigo solution + 10 c.c. solution X

required 25.45 c.c. permanganate.
" " " " 25.45 c.c. "
Average 25.45 c.c. "
20 c.c. indigo solution required 18.4 c.c. "
therefore 10 c.c. solution X required 7.05 c.c. "

The amount of gallic acid absorbed by the bone tissue was represented by 4.23 c.c. permanganate in a total of 11.28 c.c. or about 37 per cent.

SECOND EXPERIMENT.

A gallic acid solution of the same strength as the preceding was prepared, 50 c.c. were treated with 5 grms. bone tissue and 50 c.c. water [X], 50 c.c. were treated with 10 grms. bone tissue and 50 c.c. water [Y]. Another portion was diluted with exactly its own volume of water [A].

20 c.c. indigo solution + 10 c.c. solution A

required 29.6 c.c. permanganate.
" " " " 29.6 c.c. "
Average 29.6 c.c. "
20 c.c. indigo solution required 18.4 c.c. "
therefore 10 c.c. solution A. required 11.2 c.c. "

20 c.c. indigo solution + 10 c.c. solution X

required 25.35 c.c. permanganate.
" " " " 25.4 c.c. "
Average 25.37 c.c. "
20 c.c. indigo solution required 18.4 c.c. "
therefore 10 c.c. solution X required 6.97 c.c. "

20 c.c. indigo solution + 10 c.c. solution Y

required 24.1 c.c. permanganate.
" " " " 24.2 c.c. "
Average 24.15 c.c. "
20 c.c. indigo solution required 18.4 c.c. "
therefore 10 c.c. solution Y required 5.75 c.c. "

The amount of gallic acid absorbed was very nearly as in the 1st experiment, *i. e.*, 37 per cent., when the same quantity of bone tissue was used ; but reached nearly 50 per cent. when the greater quantity of bone tissue was used.

Third Experiment.

1.2517 grms. of Schuchardt's c. p. tannic acid were dissolved in one litre of water [A], 50 c.c. were treated with 5 grms. bone tissue and 50 c.c. water [X], 50 c.c. were treated with 10 grms. bone tissue and 50 c.c. water [Y]. Two parts of solution X or Y were equivalent to one of the original solution A.

20 c.c. indigo solution + 5 c.c. solution A

	required 24.7 c.c.	permanganate.
" " " "	24.95 c.c.	"
Average	24.83 c.c.	"
20 c.c. indigo solution required	18.4 c.c.	"
therefore 5 c.c. solution A "	6.43 c.c.	"

20 c.c. indigo solution + 40 c.c. solution X

	required 20.6 c.c.	permanganate.
" " " "	20.6 c.c.	"
Average	20.6 c.c.	"
20 c.c. indigo solution required	18.4 c.c.	"
therefore 40 c.c. solution X required	2.2 c.c.	"
or 10 c.c. " "	0.55 c.c.	"

20 c.c. indigo solution + 40 c.c. solution Y

	required 19.8 c.c.	permanganate.
" " " "	19.75 c.c.	"
Average	19.78 c.c.	"
20 c.c. indigo solution required	18.4 c.c.	"
therefore 40 c.c. solution Y required	1.38 c.c.	"
or 10 c.c. " " " "	0.35 c.c.	"

If the results obtained under X be taken as the amount of permanganate required by the non-tannins, we have 6.43 c.c.—0.55 c.c. =5.88 c.c.—as the amount of permanganate required by the tannin in 5 c.c. of the original solution A.

If the results obtained under Y be taken, we have 6.43 c.c.—0.35 c.c. = 6.08 c.c. Though the difference amounts to but 0.2 c.c., it is sufficient to produce a variation of nearly 4 per cent, when the results are calculated from these figures. The error, therefore, which is caused by the absorption of non-tannins, is at least as great as in Hammer's method.

Titration of the Permanganate by Oxalic Acid.

This was conducted by the method detailed in Fresenius' Quantitative Analysis: it is very accurate and calls for no further comment.

Ratio between Gallotannic Acid and Oxalic Acid.

I have already alluded to the different results which Neubauer and Councler and v. Schroeder obtained in their determinations of this ratio, and have shown how these differences may be traced to certain defects of the Loewenthal method. I do not wish to assert that my own determinations are free from these defects; on the contrary, they serve to show that varying results are unavoidable.

First Determination.

Schuchardt's c. p. tannic acid was used for these determinations. It contained, as the average of the two estimations detailed in the discussion of Hammer's method, 93.8 per cent. gallotannic acid. The oxalic acid used was purified by the method already described. 0.3936 grms. c. p. tannic acid, = 0.3515 dried at 105°, = 0.3297 pure gallotannic acid, was dissolved in 200 c.c. distilled water [A], 50 c.c. of the solution were treated with 5 grms. bone tissue, and 50 c.c. water [X]. Two parts of X were therefore equivalent to one of the original solution A.

20 c.c. indigo solution + 5 c.c. solution A

	required 26.9 c.c.	permanganate.
" " " " "	27.1 c.c.	"
Average	27.0 c.c.	"
20 c.c. indigo solution required	18.35 c.c.	"
therefore 5 c.c. solution A required	8.65 c.c.	"

20 c.c. indigo solution + 20 c.c. solution X

	required 19.4 c.c.	permanganate.
" " " " "	19.4 c.c.	"
Average	19.4 c.c.	"
20 c.c. indigo solution required	18.35 c.c.	"
therefore 20 c.c. solution X required	1.05 c.c.	"
or 10 c.c. " " "	0.52 c.c.	"

The amount of permanganate, therefore, required by the tannin in 5 c.c. of the solution A was 8.65 c.c. — 0.52 c.c. = 8.13 c.c.

Hence, 200 c.c. containing grms. 0.3297 gallotannic acid would require $\frac{8.13 \text{ cc.} \times 200}{5} = 325.2$ c.c. permanganate.

On the other hand, 0.7770 grms. pure cryst. oxalic acid required 39.7 c.c. permanganate, therefore 1000 c.c. permanganate oxidized 1.957 grms. oxalic acid; 0.6720 grms. pure cryst. oxalic acid required 34.45 c.c. permanganate, therefore 1000 c.c. permanganate oxidized 1.951 grms. oxalic acid.

Average: 1000 c.c. permanganate oxidized 1.954 grms. oxalic acid. Hence, 325.2 c.c. would oxidize 0.6354 grms. oxalic acid.

But the same quantity of permanganate was required by 0.3297 grms. gallotannic acid; therefore, 0.3297 grms. gallotannic acid were found equivalent to 0.6354 grms. oxalic acid.

From this the "oxidation ratio" is 63 parts oxalic acid to 32.67 parts gallotannic acid.

Second Determination.

0.3000 grms. c. p. tannic acid dried at 105° = 0.2814 grms. pure gallotannic acid was dissolved in 150 c.c. distilled water [A].

50 c.c. of the solution were treated with 5 grms. bone tissue, and 50 c.c. water [X]. Two parts of X were equivalent to one of the original solution A.

20 c.c. indigo solution + 5 c.c. solution A	required 27.8 c.c.	permanganate.
" " " " "	28.1 c.c.	"
Average	27.95 c.c.	"
20 c.c. indigo solution required	18.35 c.c.	"
therefore 5 c.c. solution A required	9.6 c.c.	"

20 c.c. indigo solution + 20 c.c. solution X	required 19.8 c.c.	permanganate.
" " " " "	19.8 c.c.	"
Average	19.8 c.c.	"
20 c.c. indigo solution required	18.35 c.c.	"
therefore 20 c.c. solution X required	1.45 c.c.	"
or 10 c.c. " " "	0.72 c.c.	"

The amount of permanganate required by the tannin in 5 c.c. of the orginal solution A was 9.6 c.c. — 0.72 c.c. = 8.88 c.c.

150 c.c. containing 0.2814 grms. gallotannic acid would require $\frac{8.88 \text{ c.c.} \times 150}{5} = 266.4$ c.c. permanganate. The titre of the latter was the same as in the preceding determination, hence 266.4 c.c. permanganate would oxidize 0.5205 grms. oxalic acid. Therefore, 0.2814 grms. gallotannic acid were found equivalent to 0.5205 grms. oxalic acid, whence the "oxidation ratio" was 63 parts oxalic acid to 34.06 parts gallotannic acid.

The first determination gave the ratio 63:32.67; the 2d, 63:34.06. These two determinations agree one with another much better than do those of Neubauer with those of Councler and v. Schroeder; but it must be observed that the former were made as nearly as possible under identical conditions, and that the same gallotannic acid was used in both of my determinations. Had the conditions been varied in any of the particulars already discussed, such as the rate of adding the permanganate, quantity of bone tissue used for absorption, etc., I have little doubt but that the results would have been more divergent.

It may, in fact, be fairly asserted that no determination of the "oxidation ratio" between gallotannic acid and oxalic acid is to be looked upon as reliable and final.

It is evident that a definite quantity of gallotannic acid will require for its oxidation into carbonic acid and water an equally definite quantity of oxygen. One molecule of gallotannic acid (mol. weight = 322) will require 24 atoms of oxygen.

$$C_{14}H_{10}O_9 + 24\ O = 14\ CO_2 + 5\ H_2O.$$

One molecule of oxalic acid (mol. weight = 126) requires 1 atom of oxygen.

$$(C_2H_2O_4 + 2\ \text{Aq.}) + O = 2\ CO_2 + 3\ H_2O.$$

Consequently, 322 parts of gallotannic acid would require the same amount of oxygen or of permanganate as $24 \times 126 = 3064$ parts of oxalic acid, and therefore 63 parts of the latter be equivalent to 6.62 parts of the former. These figures in no way accord with the "oxidation ratio" as determined by Loewenthal's method, proving that under the conditions of that method the oxidation of tannin is anything but complete. It is probable that one or several intermediate products of oxidation are formed, of which we have, however, no knowledge.

It may fairly be presumed that tannins which differ in chemical composition, would be differently affected by oxidizing agents. Thus, even if the exact ratio between gallotannic acid and oxalic acid could be established, it would not apply to other tannins. Indeed, Oser, who attempted the determination of the "oxidation ratio" for the tannin of oak bark, found it as 63 parts oxalic acid to 62.32 oak tannin. But Simand made it 63:60.11.

Comparison of the Methods of Estimating Tannins.

Up to the present time over thirty such methods have been devised, of which the great majority have not even a superficial claim to being reliable. In this category must be included the numerous methods based on precipitation of tannins with metallic bases. Every now and then some new method is announced, which simply repeats the faults of those already known. While I have pointed out the defects to which Hammer's and Loewenthal's methods are liable, I have not intended to imply that they are less trustworthy than other methods; on the contrary, it is because they appeared based on sounder principles, that they merited a closer investigation.

I am forced, however, to believe that the defects peculiar to Loewenthal's method are not to be remedied until the reaction of permanganate on the various tannins under the conditions of that method is perfectly explained. Hammer's method is certainly the most reliable, as it is exposed to but a single serious error, viz.: the absorption of non-tannins by gelatine producing tissue. If this error is avoided or corrected, Hammer's method will become sufficiently accurate for all the purposes of commercial analysis. I have recently devised a modification of the method by which the error alluded to is at least greatly diminished, and with certain analytical data will make it the subject of a future communication.