from that of the analysis proper. Exp. 20–22 are three comparative analyses from the writer's note-book.

TABLE VI.

COPPER ANALYSIS.

		Per cent. sulphur.		Per cent. sulphur.
20	Direct precipitation	0.0000	Author's method	0.0023
21	" "	0.6000	" "	0.6500
22	" "	0 to 0.0050	" "	(1) 0.0112
22	Chlorine method	0.0189?		(2) 0.0094

It is evident that the method just detailed, is extremely well adapted to the analysis of refined copper.

The sulphur in the metal is brought into solution, and finally precipitated in a pure condition without loss, and a blank analysis is possible under the same conditions. The difference between the two results, expresses the true quantity of sulphur present.

Another chemist stated sometime ago that he, also, had been obliged to try a similar plan. The author has, however, worked out the foregoing process in its improved form independently, and in presenting this standard method, desires to express his indebtedness to the gentleman who has so kindly consented to read the paper before the assembly.

ACIDIMETRIC ESTIMATION OF VEGETABLE ALKALOIDS. A STUDY OF INDICATORS.[1]

BY LYMAN F. KEBLER.

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THE titration of alkaloids with volumetric acid solutions has been evolved from the study of the basicity of the alkaloids on the one hand, and from their behavior with indicators on the other. The method appears to have been developed somewhat spasmodically from quite an early period. As early as 1846 M. Schlössing[2] proposed the method and applied it to the titration of nicotine with a view of establishing its equivalent; using sulphuric acid and litmus in his work. Sixteen

[1] Read at the Springfield meeting.

[2] *1847, Comp. rend.*, **23**, 1142; *1847, Ann. Chim. Phys.* [3], **19**, 230; Chem. Gaz., 5, 41; Am. J. Pharm., 19, 68.

years later the work was taken up by Wittstein,[1] who was followed by F. M. Brandl,[2] Liecke,[3] Kosutany,[4] and G. Dragendorff.[5] Up to this time nicotine and conine were the only alkaloids operated on, and litmus the only indicator employed. In 1879 L. van Itallie[6] extended the work to several other alkaloids, using lacmöid as indicator. A. W. Gerrard[7] a few years later employed litmus and phenolphthalein in titrating the alkaloids of belladonna. From the contributions of O. Schweissinger,[8] who used cochineal as indicator, and those of E. Dieterich[9] and P. C. Plugge[10] we may ascribe the impetus which the titration of alkaloids with volumetric acid solutions received at the beginning of the present decade.

The method had been gaining ground rapidly when several most valuable communications appeared by C. C. Keller,[11] of Zürich, since which great improvement has been made.

In volumetric analysis, the first question demanding attention is a suitable indicator or delicate end reaction.[12] The object of this communication is to present the results of a study of five indicators in titrating alkaloids, thinking perhaps it may be of some service in formulating systematized methods of analysis in alkaloidal chemistry. The discordant results of analysis often obtained by different chemists operating on the same sample are greatly to be regretted. It is the writer's opinion that

1 *1862*, Vierjahrschr. prakt. Pharm., 11, 351.

2 *1864*, Vierjahrschr. prakt. Pharm., 13, 322.

3 *1865*, Mittheilungen des hannov. Gew-Ver., p. 160; Ding. poly. J., 178, 235; Polyt. Notizbl., No. 20; Ztschr. anal Chem., 4, 492.

4 *1873*, Kosutany, Anal. Bestim. einiger Bestandth. d Tabakspflanze. Diss. Altenburg, Hungary.

5 *1874*, Chem. Werthbestim., p. 42 and 55; see also Plant Analyses. *1884*, Eng. Ed., pages 63 and 188.

6 *1879*, Nederland. Tydschr. v. Pharm., Jan; *Analyst*, 14, 118.

7 *1882* and *1884*, Year-Book of Pharm., p. 401, 447.

8 *1886*, Pharm. Centralhalle, 27, 492.

9 *1887*, Pharm. Centralhalle, 28, 21; Pharm. J. Trans. [3], 17, 888; *Am. J. Pharm.*, 59, 179.

10 *1887*, Arch. d. Pharm. [3], 25, 45, 49; J. de Pharm. et de Chim. [5], 15, 571; *Ber. d. chem. Ges.*, 20, 148; *J. Chem. Soc.*, 52, 621.

11 *1892*, Schweïz., Wochenschr. f. Chem. u. Pharm., 30, 501, 509; *Am. J. Pharm.*, 65, 78. *1883*, Schweïz., Wochenschr. f. Chem. u. Pharm., 31, 473; Ztschr. Oesterreich- Apoteker., 47, 563, 586; *Am. J. Pharm.*, 66, 42.

12 Alkaloids, generally, are neutral to phenolphthalein, consequently it cannot be employed in titrating alkaloids directly. It is available for indirect titrations, *i. e.*, estimating the amount of acids combined with an alkaloid in its neutral salts.

the discrepancies are chiefly due to differences in *modus operandi*, to defective apparatus, and, in volumetric analysis, to different end reaction tints arbitrarily assumed by each worker.

In order to eliminate the factors of uncertainty as completely as possible the methods of operation were carefully written out and closely adhered to in all the work. The burettes and a pipette were carefully calibrated in order to ascertain the necessary factor for correction. The method of calibration was as follows: each burette and pipette was exactly filled to the zero mark with distilled water, at 15° C. and ten cc., delivered into a tared weighing flask and weighed, then the next ten cc. were treated in the same manner, and so on until the entire capacity of each was tested. A glass-stoppered cylinder was also standardized. All efforts to standardize a liter flask were thwarted. A large balance sufficiently sensitive to do the work satisfactorily could not be found.

In titration the personal equation plays an important part. Authorities are not agreed on end reaction tints, each operator relying on his own judgment. The writer thinks it correct to titrate to the point where a different color from the initial color is developed. In order to obtain standard end reaction tints for alkaloids it will be necessary to prepare some absolutely pure alkaloid; treat a molecular quantity of the alkaloid with an equivalent of the acid in question to form a neutral salt, then add one drop more of the decinormal acid for an acid color reaction. For alkaline tints add one drop of the centinormal alkaline solution to a solution of neutral alkaloidal salt, theoretically prepared.

In this work the writer titrated from acid to alkaline solutions as follows: Brazil wood, from yellow to onion-red, the purple ultimately fading to this; cochineal from yellow to bluish-red; haematoxylin from yellow to brown-orange; litmus from red to onion-red, and methyl orange from red to straw-yellow.

The indicator solutions were prepared according to the most approved processes. Cochineal and litmus were prepared according to the specifications of Sutton's Volumetric Analysis, sixth edition. Phenolphthalein, one gram dissolved in one liter of fifty per cent. alcohol. Haematoxylin, well crystallized, one

gram dissolved in 100 cc. of strong alcohol. The method best suited for preparing the Brazil wood solution, is to place three grams of the wood into a casserole, add ten cc. of distilled water, boil gently for a few minutes, cool, and filter. A freshly prepared solution has given the writer the most satisfactory results. Methyl orange, one gram dissolved in one liter of distilled water. Considerable difficulty was experienced in obtaining even a fairly satisfactory product of methyl orange. The method proposed by Mr. B. Reinitzer[1] for preparing the litmus solution did not come to the writer's notice until considerable work had been done with the solution prepared as above.

In titration the following quantities of the several indicators were employed: methyl orange, Brazil wood, cochineal, and phenolphthalein, five drops each; litmus ten drops and haematoxylin three drops.

The standard solution employed in this investigation, from which the exact strength of the other volumetric solution was determined, was a solution of normal sulphuric acid. This solution was prepared from data obtained by the several methods; titration against pure anhydrous sodium carbonate, using the above indicators; precipitation as barium sulphate and Weinig's[2] process. After some experimentation, it was found that Weinig's method gave the most satisfactory results. The method is simple and yields very concordant results. The following are the data obtained from an approximately normal sulphuric acid solution with the above methods:

Indicators and methods.	No. of cc. of acid solution required per ten cc. of normal sodium carbonate.	Grams of SO_3 in ten cc. of the acid solution.
Brazil wood	9.50	0.4211
Haematoxylin	9.54	0.4192
Cochineal	9.50	0.4211
Litmus	9.50	0.4211
Methyl orange	9.50	0.4211
Phenolphthalein	9.45	0.4216
Weinig's method	....	0.4247
Barium sulphate method	....	0.4200

Due precaution was taken to boil the solution thoroughly with

1 *1894*, Ztschr. angew. Chem., 547, 573; Chem. News, 70, 225, 239, 249.
2 *1892*, Ztschr. angew. Chem., 204; Analyst, 17, 99.

the indicators requiring it. With solutions of the above strength it was impossible to detect any difference in the sensitiveness of most of the indicators.

With the normal sulphuric acid solution a normal solution of pure potassium hydroxide was standardized. From the normal sulphuric acid solution and normal alkaline solution there were prepared, respectively, a decinormal acid solution and a centinormal alkaline solution. The two solutions thus prepared were carefully titrated against each other, employing the above indicators with the following results:

Indicators.	No. of cc. of normal sulphuric acid.	No. of cc. of centinormal KOH required per ten cc. of decinormal H_2SO_4.	
		LaWall.	Kebler.
Phenolphthalein	10	101.80	102.00
Brazil wood	10	99.56	100.00
Cochineal	10	100.58	99.80
Haematoxylin	10	99.76	100.00
Litmus	10	99.97	99.60
Methyl orange	10	92.67	98.53

My associate, Mr. LaWall, took up a portion of the work, which he executed independently, using, however, the same solutions and apparatus that the writer employed. The above, and all subsequent results, are the average of duplicate, triplicate or more titrations.

The titration of pure alkaloids, as found in the market, was next undertaken. With quinine and codeine the following method was used: two grams of the alkaloid were placed in the cylinder, dissolved in alcohol, and diluted up to 100 cc. with alcohol. To ten cc. of this solution and the requisite quantity of indicator contained in a suitable beaker, the decinormal acid solution was added to slight excess, agitated, allowed to stand a few minutes, the sides of the beaker well washed down with distilled water, adding about forty cc., and the excess of acid titrated back with the centinormal alkaline solution.

With alkaloids not freely soluble in alcohol, the following procedure was adopted: two grams of the alkaloid were placed into a 200 cc. beaker, seventy-five cc. of decinormal acid added, the contents of the beaker warmed on a water-bath and occasionally agitated until the alkaloid was dissolved. The beaker

and contents were then cooled, the contents transferred to a 100 cc. cylinder, the beaker carefully rinsed with several successive portions of water, transferred to the 100 cc. cylinder and finally made up to 100 cc. with water. Each ten cc. contained two-tenths of a gram of alkaloid and seven and a half cc. of decinormal acid solution. After adding the requisite amount of indicator to ten cc. of the alkaloidal solution and diluting up to about fifty cc., the excess of acid was carefully retitrated. Two or more titrations were made in every case, with the same solution and indicator, by adding to the solution just finished, another portion of the decinormal acid solution and retitrating with the centinormal alkaline solution, taking finally the average reading.

The above methods of titration and preparation of solutions were employed with several pure alkaloids. The results are tabulated below.

	Quinine.		Strychnine.	Morphine.	Codeine.
Indicators.	LaWall.	Kebler.	Kebler.	Kebler.	Kebler.
Brazil wood.....	99.90	101.97	99.36	98.93	95.75
Cochineal	105.56	102.54	103.20	99.08	97.09
Haematoxylin	99.81	103.37	100.03	98.17	95.90
Litmus	101.80	103.55	103.54	98.93	96.38
Methyl orange.....	....	123.27	104.21	100.59	98.11

The number of times the analyst is requested to investigate the purity of refined alkaloids is comparatively small, but the crude alkaloids claim a greater share of his time and attention.

The next step was to investigate the adaptability of the above process to crude morphine and crude cocaine. The results are as follows:

	Crude morphine.		Crude cocaine.
Indicators.	LaWall.	Kebler.	Kebler.
Brazil wood.....	99.23	98.47	95.90
Cochineal.....	100.14	99.53	97.11
Haematoxylin	99.08	97.59	95.74
Litmus	99.50	98.93	96.82
Methyl orange.....	102.10	100.02	100.14

With the same crude morphine the ash method yielded 97.59 per cent., the lime-water method 98.22 per cent., and the absolute alcohol method 98.33 per cent. of pure morphine.

A complete analysis was made of the crude cocaine to ascer-

tain how nearly the titrations corresponded with the gravimetric process of Dr. Squibb.[1]

	Per cent.
Moisture	0.405
Cocaine nearly pure	97.300
Material soluble in ether	0.100
Material insoluble in ether	1.810
Loss	0.385
Total	100.00

Notwithstanding the fact that crude alkaloids claim considerable attention on the part of the analyst, yet only a few are found already extracted on the market. It generally happens that the operator is requested not only to determine the amount of pure alkaloids, but also to extract them from their natural sources. For this purpose the writer employed a modification of Keller's process. The method is as follows: place ten grams of the dry drug into a 250 cc. flask, add twenty-five grams of chloroform, seventy-five grams of ether, stopper the flask securely, agitate well for several minutes, add ten grams of ten per cent. ammonia water, then agitate frequently and during one hour. On adding five grams more of ten per cent. ammonia water and shaking well, the suspended powder agglutinates into a lump, the liquid becomes clear, after standing a few minutes, and can be poured off almost completely.

1. When the mixture has completely separated, pour off fifty grams into a beaker, evaporate the solvent on a water-bath, add ten cc. of ether, and evaporate again. Dissolve the varnish-like residue in fifteen cc. of alcohol, with heat, add water to slight permanent turbidity, the requisite quantity of indicator and an excess of the acid solution; retitrate with the centinormal alkaline solution.

2. When the mixture has completely separated pour fifty grams into a separatory funnel, treat at once with twenty cc. of acidulated water. After thorough agitation and complete separation remove the aqueous solution into a second separatory funnel. Repeat the above operation twice more successively with fifteen cc. of slightly acidulated water. The acidulated water

[1] Ephemeris, 3, 1171.

in the second separatory funnel is rendered alkaline with ammonia water, the alkaloid removed successively with twenty cc., fifteen cc., and fifteen cc. of a mixture of three parts (by volume) of chloroform and one part of ether. Collect the chloroform-ether mixture in a tared beaker and distil off the solvent. The varnish-like residue is twice treated with eight cc. of ether, evaporated on a water-bath and dried to constant weight on the water-bath. The varnish-like residue is next dissolved in fifteen cc. of alcohol and treated as in (1) above.

Nux vomica and ipecac root were treated according to processes (1) and (2); belladonna leaves according to process (2). The results are as follows:

	Per cent. of alkaloids in nux vomica by process (1).		Per cent. of alkaloids in nux vomica by process (2). Gravimetrically.		Per cent. of alkaloids in nux vomica by process (2). Volumetrically.		Per cent. of alkaloid in ipecac root by process (1.)	
	LaWall.	Kebler.	LaWall.	Kebler.	LaWall.	Kebler.	LaWall.	Kebler
Brazil wood.....	2.04	2.58	2.94	3.00	2.37	2.37	2.46	2.54
Cochineal	2.64	2.69	2.86	3.10	2.42	2.39	2.59	2.49
Haematoxylin ...	2.18	2.24	2.88	3.11	2.23	2.27	2.48	2.54
Litmus	2.38	2.34	2.93	3.05	2.55	2.37	2.55	2.57
Methyl orange...	3.02	3.64	2.93	3.02	2.65	2.61	2.95	3.30

	Per cent. of alkaloid in ipecac root by process (2). Gravimetrically.		Per cent. of alkaloid in ipecac root by process (2). Volumetrically.		Per cent. of alkaloids in belladonna leaves by process (2). Gravimetrically.		Per cent. of alkaloids in belladonna leaves by process (2). Volumetrically.	
	LaWall.	Kebler.	LaWall.	Kebler.	LaWall.	Kebler.	LaWall.	Kebler.
Brazil wood.....	2.58	2.60	2.36	2.35	0.26	0.20	0.19	0.15
Cochineal	2.63	2.68	2.52	2.33	0.28	0.20	0.24	0.14
Haematoxylin ...	2.58	2.68	2.35	2.33	0.27	0.22	0.21	0.13
Litmus	2.62	2.60	2.40	2.25	0.24	0.18	0.20	0.15
Methyl orange...	2.66	2.63	2.89	2.61	0.25	0.20	0.23	0.20

According to the well-established method of Messrs. Dunstan[1] and Short, the nux vomica examined contained 2.89 per cent. of crude alkaloid. On carefully titrating this crude product with a volumetric acid solution, 2.12 per cent. of pure alkaloid was indicated. Cochineal was used as an indicator. These figures show that this method produces an alkaloid residue containing a smaller percentage of pure alkaloid than that obtained by Keller's process.

[1] *1883*, Pharm. J. Trans. [3], **13**, 665.

From the results embodied in this paper it can safely be concluded that methyl orange cannot be numbered with the indicators suitable for titrating alkaloids. With centinormal, fifth decinormal and other solutions of various strengths it fails to give satisfactory results. Notwithstanding the sensitiveness claimed for it, the writer believes that its days, as an ideal indicator, are numbered. Even Professor Lunge, the staunch advocate of methyl orange, has admitted that a properly prepared solution of litmus is quite superior to this indicator, in inorganic estimations.

A solution of litmus prepared according to the directions herein employed is quite unsatisfactory for delicate titrations. The method proposed by Reinitzer promises to be better suited.

Of the indicators thus far considered, haematoxylin, Brazil wood, and cochineal give very promising results. Haematoxylin justly claims first place and Brazil wood the second. Other indicators will be considered in due time.

As stated above, the prime object of this investigation is to ascertain what indicators are best adapted to the titration of alkaloids; but in order to determine how reliable the results were, gravimetric determinations necessarily formed a part of the work.

When it is remembered that not only do analytical methods contain inherent limitations, but also that each operator possesses a positive or a negative equation of error, the reader will undoubtedly concur with the writer that the results are very satisfactory. Attention must again be called to the fact that the work was conducted under precisely the same conditions.

As would naturally be expected, the amount of alkaloid obtained by process (2) is smaller than that secured by process (1). A small per cent. of the alkaloid may be lost during the process of extraction. The small amount of coloring-matter possibly vitiates the results, or perhaps some non-alkaloidal substance increases the yield in process (2).

From the hundreds of assays made by the author, he feels justified in stating that all of the gravimetric processes yield products containing considerable non-alkaloidal matter, and hopes that the day is not far distant when all gravimetric results

will at least be supplemented by volumetric methods, if not displaced by them.

THE MORE VALUABLE LITERATURE ON INDICATORS DURING THE LAST TWO DECADES.

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