THE TITRATION OF CERTAIN ALKALOIDS.

BY M. G. MELLON AND J. TIGELAAR.

For the titrametric determination of the alkaloids atropine, brucine and strychnine, through the addition of an excess of standard acid and the back titration with a base, the edition of 1925 of the U. S. Pharmacopœia specifies the use of methyl red as an indicator.

Since the date of compiling most of the material for the last edition of these standard methods a number of investigations have been published[1] dealing with the determination of various alkaloids by means of several methods. On the basis of the data presented various individuals have drawn quite different conclusions regarding the indicators which should be used in making volumetric titrations. Because of this disagreement Dean C. B. Jordan[2] suggested to the writers that determinations be made of certain of the alkaloids with the idea of trying some of the different procedures proposed in order to determine whether any recommendation for a change should be made in the forthcoming edition of the Pharmacopœia.

The work undertaken had the following objectives: to check the ordinary volumetric method with the potentiometric method using a quinhydrone electrode; and to run the volumetric method using both the indicators recommended by previous investigators and any others available whose p_H ranges seemed likely to be usable. The alkaloids selected were atropine, brucine and strychnine.

EXPERIMENTAL WORK.

The alkaloids were in the form of commercially prepared white solids or in the form of chloroform or chloroform-ether extracts prepared in the local School of Pharmacy according to the specifications of the U. S. Pharmacopœia. Solutions of approximately 0.1 *N* hydrochloric and sulphuric acids and 0.0200 *N* sodium hydroxide were prepared with the usual analytical precautions. The solutions of the indicators were prepared according to the directions of Clark.[3] A solution of modified methyl red was made by dissolving 1.25 Gm. of methyl red and 0.825 Gm. of methylene blue in a liter of 90% ethanol.[4]

For the potentiometric titration a L. and N. student potentiometer was used together with normal calomel and quinhydrone electrodes connected with a salt bridge containing potassium chloride.

For making titrations a weighed sample was dissolved in the smallest possible

[1] J. Evers, JOUR. A. PH. A., 10 (1921), 676; Herzig, *Arch. Pharm.* (1921), 249, 259, 308; Holt and Kahlenberg, JOUR. A. PH. A., 20 (1931), 11; Kolthoff, *Bilochem. Z.*, 162 (1925), 289, JOUR. A. PH. A., 14 (1925), 298, *Pharm. Weekblad*, 62 (1925), 287, 478, *Z. anorg. allgem. Chem.*, 112 (1920), 196; Krantz, JOUR. A. PH. A., 14 (1925), 294, 19 (1930), 1299; Maricq, *Bull. soc. chim. Belg.*, 38 (1929), 265, 426; Masucci and Moffat, JOUR. A. PH. A., 12 (1923), 609; McGill, *J. Am. Chem. Soc.*, 44 (1922), 2156, JOUR. A. PH. A., 11 (1922), 1003; North and Beal, JOUR. A. PH. A., 13 (1924), 889, 1001; Popoff and McHenry, *Ind. Eng. Chem.*, 20 (1928), 534, JOUR. A. PH. A., 14 (1925), 473; Prideaux and Winfield, *Analyst*, 55 (1930), 561; Rasmussen and Schou, *Z. Electrochem.*, 31 (1925), 189; Treadwell and Janet, *Helv. Chim. Acta*, 6 (1923), 734; Wagener and McGill, JOUR. A. PH. A., 14 (1925), 288; Wales, *Ind. Eng. Chem.*, 18 (1926), 390.

[2] School of Pharmacy, Purdue University; Chairman of Sub-Committee on Proximate Assays of the Committee of Revision of the U. S. Pharmacopœia.

[3] Clark, "Determination of Hydrogen Ions" (1928), 92, 94.

[4] Johnson and Green, *Ind. Eng. Chem., Anal. Ed.*, 2 (1930), 2.

amount of neutral, 95% ethanol or directly in the standard acid. About 1 ml. more than the calculated equivalent amount of acid was added and the solution diluted to about 75 ml. After adding one or two drops of the desired indicator, the solution was titrated back with the standard base to the proper end-point. From the actual and the theoretical amount of acid required for the formation of the alkaloidal salt the percentage purity was determined. This value depends both upon the p_H ranges of the different indicators and the actual color change selected by the analyst to mark the end-point. In several cases both hydrochloric and sulphuric acids were used but with no more variation than might be expected from the experimental error. The mean value calculated for the percentage purity is the average of three to twelve separate determinations. Samples of approximately 0.4 Gm. were used.

The results obtained for the alkaloids atropine, brucine and strychnine are shown in Table I.

TABLE I.—RESULTS OBTAINED IN THE TITRATION OF VARIOUS ALKALOIDS USING DIFFERENT INDICATORS.

Indicator.	p_H Range.	Mean Value for Purity Per Cent.	Variation from Mean, Per Cent.
	Atropine.		
Brom phenol blue	3.0–4.6	99.57	±0.20
Brom cresol green	4.0–5.6	98.73	0.30
Methyl red	4.4–6.0	98.67	0.10
Modified methyl red	4.4–6.0	98.70	0.11
p-Nitrophenol	5.0–7.0	98.39	0.11
Brom cresol purple	5.2–6.8	98.51	0.12
	Brucine.		
Brom phenol blue	3.0–4.6	98.51	0.09
Brom cresol green	4.0–5.6	97.76	0.10
Methyl red	4.4–6.0	96.96	0.06
Modified methyl red	4.4–6.0	97.03	0.18
p-Nitrophenol	5.0–7.0	96.85	0.09
Chlor phenol red	5.0–6.6	96.49	0.16
Brom cresol purple	5.2–6.8	96.36	0.08
	Strychnine.		
Brom phenol blue	3.0–4.6	99.45	0.30
Methyl red	4.4–6.0	98.40	0.14
Modified methyl red	4.4–6.0	98.45	0.10
Chlor phenol red	5.0–6.6	98.03	0.09
Brom cresol purple	5.2–6.8	97.81	0.16

Atropine was titrated as soon as possible after being dissolved. No heat was applied to aid the process of solution as such procedure rendered checking titrations difficult to obtain. Kolthoff[1] called attention to the instability of such a solution.

In attempting to titrate colchicine indigo carmine was used as an indicator. The color change was not sharp and the amount of alkali required in the back titration indicated that an alkaloidal salt was not formed with the acid, thus confirming the reports of others on this point.

[1] *Biochem. Z.*, 162 (1925), 289.

All of the above results were obtained on commercial material which was practically white. Since it seemed desirable to determine whether determinations could be made with indicators on material not so well purified, samples were titrated which had been made by students by extracting crude nux vomica and belladonna following the method of the U. S. Pharmacopœia. The solutions were more or less yellowish in hue. In both cases the end-points with modified methyl red were distinct, the results agreeing among themselves for each alkaloid within about five parts per thousand. With belladonna the color changes with brom cresol green and brom cresol purple were distinct, but *p*-nitrophenol was useless.

Following essentially the procedure of Rasmussen and Schou[1] potentiometric determinations were run by dissolving the commercial alkaloid in a small excess of standard acid, diluting to about 100 ml. and titrating back with sodium hydroxide, using normal calomel and quinhydrone electrodes. Plotting the p_H values as ordinates and the volume of base as abscissas, the results are shown in Fig. 1. The results in each case are the average of about seven determinations, the deviation from the mean being not more than 0.1 p_H units. In the curves the chief items of interest are the location, the slope and the length of the nearly vertical portions.

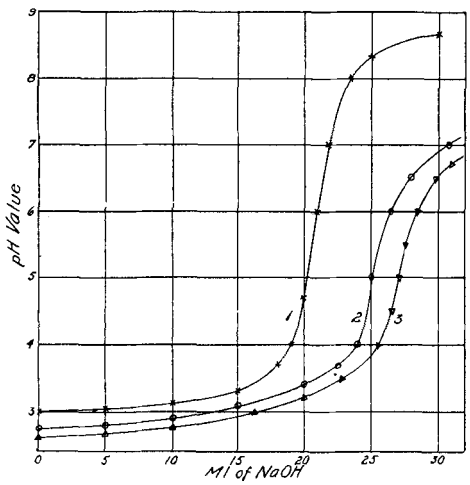

Fig. 1.—Curves for the titration of (1) atropine, (2) strychnine and (3) brucine.

DISCUSSION AND SUMMARY.

Being free from interference by color in the solutions titrated and being largely independent of the judgment of the analyst as to where the end-point occurs, potentiometric determinations are probably more generally applicable; but for commercial analyses the method is not as simple and rapid as the usual volumetric procedure. The latter is preferable, therefore, as long as the results obtained lie within reasonable limits of precision.

An inspection of the curves shown in Fig. 1 indicates the approximate transformation range indicators should have to be suitable for the titration of the three alkaloids studied. Taking the mid-way point of the nearly vertical portion of the curves, the end-point would be for atropine at a p_H value of about 6 and for brucine and strychnine about 5. As far as their transformation ranges are concerned, methyl red and brom cresol green seem to be the best for brucine and strychnine and brom cresol purple for atropine. As all the indicators mentioned in Table I except modified methyl red have yellow as either their acidic or basic color, they have a common defect if the solution to be titrated is itself yellowish. Although methyl red has not quite the best p_H range for the titration of atropine, the error involved in its use in determining the small amount of active constituent present in crude drugs would be small.

[1] *Z. Elektrochem.*, 31 (1925), 189.

The divergence of results shown in Table I for any one alkaloid indicates the desirability of specifying some one indicator if concordant values are to be obtained. The variations shown are to be explained presumably by one or both of the following factors: the different transformation ranges of the indicators, and the actual hue selected by the analyst to mark the attainment of the end-point of the titration. In the latter connection, modified methyl red has a distinct advantage in having near the center of its transformation range a more or less neutral hue which may be taken as the end-point.

In view of the satisfaction which modified methyl red has given in this Laboratory for any titrations in which methyl red is applicable, and in view of the advantage which it seems to possess for the titration of the alkaloids studied, the writers recommend that it be specified as the preferable indicator for use in the determination of atropine, brucine and strychnine. The potentiometric method, using a quinhydrone electrode, should be considered as an alternative procedure.

DEPARTMENT OF CHEMISTRY,
PURDUE UNIVERSITY,
LAFAYETTE, INDIANA.

MICROBIOLOGY VERSUS BACTERIOLOGY.*

BY FANCHON HART.

The term "microbiology" was adopted because it represents most adequately the study of those biologicals concerned with micro-organisms. We might have called the subject biological materia medica, but that would not differentiate it from the study of the glandular products used in medicine.

While an outline of the course would seem to indicate that the topics under consideration are similar to those of Applied Bacteriology, the subject matter, mode of presentation, as well as the methods to be used should be the factors which bear out the objectives of the curriculum.

Microbiology has found its way into the pharmacy curriculum because a knowledge of the subject has become as essential to the pharmacist as an applied course in bacteriology is for the medical student. And right here I would like to state my disapproval of the introduction of a medical course in bacteriology for those intending to minister to the public needs in the sole capacity of retail pharmacist. To my knowledge, the practicing pharmacist is not called upon to make diagnostic tests. He has neither the equipment nor background for this type of technical work. The Board of Health of New York City clearly recognizes this fact in that it not only demands a B.S., but in addition, requires that the applicant, for a permit to undertake this work, show his ability through a searching and specific examination subsequent to an inspection of his laboratory.

On the other hand, preventive medicine, rather than therapeutic medicine is making itself felt, and even growing in use and appreciation as the public becomes more aware of its rightful share of good health and wholesome living conditions. The physician, with rare exceptions, no longer meets with opposition at the suggestion of prophylactic treatment for the exposed members of the family;

* Scientific Section, A. PH. A., Miami meeting, 1931.