2. The interference of organic compounds with Mandelin's test for strychnine have been determined when present in small and large amounts.

3. In toxicological work too much dependence should not be placed in the color reactions for strychnine. The test should be checked by the crystalline form, taste and physiological action.

REFERENCES.

(1) Mandelin, *Phar. Zeit. für Russland*, 22 (1883), 345; *Jahr. der Pharm., Pharmacog. und Toxicologie* (1884), 766.
(2) Witthaus, "Manual of Toxicology," 2nd Edition (1911), 1056–1067.
(3) P. A. W. Self, *Pharm. J.*, 94 (1915), 384, 419.
(4) Kundrat, *Z. anal. Chem.*, 28 (1889), 709; *Chem.-Ztg.*, 13, 265.
(5) Efisio Mameli, *Gazz. chim. ital.*, 41 (1911), 329.
(6) Efisio Mameli, *Boll. chim.-farm.*, 53 (1914), 366.

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A POTENTIOMETRIC ASSAY OF CINCHONA.

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INTRODUCTION.

The assays of cinchona bark and its preparations have been studied comprehensively by various investigators. A very complete survey of the various methods of evaluating this drug is given by Dubreuil (1). This investigator favors the iodometric method of estimating the alkaloidal residue, as he claims it gives the most satisfactory results. McGill (2) applied electrical titration methods to the assay of this drug and obtained concordant results. The same method was applied to nux vomica and belladonna by McGill and Wagener (3) with an equal degree of success. In this work McGill and his associates (4) were able to eliminate the shaking out process. Very recently Maricq (5) applied the potentiometric titration to the estimation of alkaloidal residues. An excess of a solution of mercuric hydrogen iodide is added and the alkaloid is precipitated with the liberation of an equivalent quantity of hydrogen iodide. In a portion of the filtrate the hydrogen iodide is estimated potentiometrically using a mercuric chloride solution.

In a previous communication to THIS JOURNAL, the author (6) showed that it was possible to determine the quantity of alkaloid present in a solution, the hydrogen-ion concentration of the solution having been determined. From this datum the amount of alkaloid present was calculated.

It is the purpose of this present investigation to apply this method to the evaluation of cinchona alkaloid residues.

EXPERIMENTAL.

Method of Preparing Graph.—In the Pharmacopœial assay of cinchona bark the final extraction of alkaloids represents 4 Gm. of drug. A drug containing 7 per cent of alkaloids would yield 280 mg. of alkaloids. Using 0.0309 as the volumetric equivalent of these alkaloids in terms of 0.1*N* acid, 9.06 cc. of acid would

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be required to combine chemically with the alkaloids. Accordingly, 280 mg. of purified alkaloids were dissolved in this volume of acid and the solution made up to 100 cc.

Keeping the quantities of acid constant other solutions were prepared varying the quantities of alkaloid to correspond to different percentages of alkaloid present in the drug. The p_H of all of these solutions was measured electrometrically using a hydrogen electrode, Wilson (7) type. The following table shows the results of these measurements:

TABLE I.

No.	Percentage of alkaloid.	p_H of solution.
1	7.0	5.90
2	6.5	4.89
3	6.0	4.55
4	5.5	4.35
5	5.0	4.10
6	4.5	3.79
7	4.0	3.50
8	3.5	3.15
9	3.0	2.76
10	2.5	2.50

From these data a graph was constructed using the percentages of alkaloids in the drug as abscissæ and the p_H of the solutions as ordinates.

Obviously the reliability of this method of assaying cinochona would depend upon whether or not the p_H of these solutions was affected by the amounts of the different alkaloids of the drug varying. This possibility was tested by dissolving the various alkaloids in 9.06 cc. of 0.1*N* acid and determining the p_H of the solutions.

TABLE II.

No.	Proportions of alkaloids.		Amount of acid. 0.1*N*.	p_H.
1	Mixed alkaloids	200 mg.	6.06 cc.	4.12
2	Cinchonine	50 mg.		
	Cinchonidine	50 mg.		
	Quinine	50 mg.	9.06 cc.	4.05
	Quinidine	50 mg		
		200 mg.		
3	Cinchonine	75 mg.		
	Cinchonidine	25 mg.		
	Quinine	50 mg.	9.06 cc.	4.10
	Quinidine	50 mg.		
		200 mg.		
4	Cinchonine	50 mg.		
	Cinchonidine	50 mg.		
	Quinine	75 mg.	9.06 cc.	4.10
	Quinidine	25 mg.		
		200 mg.		

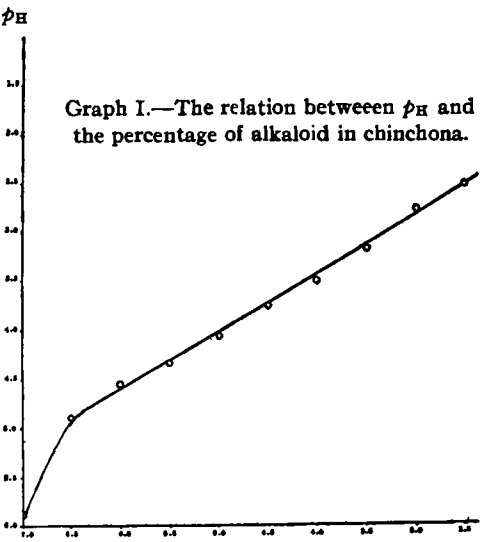
These results indicate that the p_H of the solution is inappreciably affected by varying the proportions of the alkaloids, but on the other hand it is a function of the entire quantity present.

Kolthoff (8) in the discussion of the author's former paper suggested the possibility of alkaloidal reduction by the hydrogen in the electrode, thus vitiating the results. Also Popoff and McHenry (9) suggest that McGill's results on the titration of cinchona were lower than the Pharmacopœial values due to alkaloidal reduction.

TABLE III.

No. of solution.	p_H with quinhydrone electrode.
1	4.11
2	4.06
3	4.12
4	4.14

By prolonged contact of these solutions with the hydrogen of the electrode catalyzed by the platinum black, the author has noticed slight fluctuations of the light on the galvanometer scale, which is possibly due to an oxidation-reduction potential. In order to determine whether or not this vitiated the results of the determination, the p_H of the foregoing solutions was determined by means of the quinhydrone electrode. Table III records these results. The electrode immersed in $M/20$ potassium acid phthalate solution at 18° C. showed this solution to have a p_H of 4.00.



Graph I.—The relation betweeen p_H and the percentage of alkaloid in chinchona.

These results seem to indicate that concordant results can be obtained by means of the hydrogen or quinhydrone electrodes.

Estimation of Cinchona Alkaloids.—A sample of unpurified mixed cinchona alkaloids was obtained from the drug and three samples were accurately weighed. Each was dissolved in a small quantity of neutral ether, 9.06 cc. of $0.1N$ hydrochloric acid added and the ether slowly volatilized leaving the alkaloids dissolved in the acid. These were made up to 100 cc., the p_H determined. The amounts of alkaloids were determined from the graph.

TABLE IV.

No.	Gm. present.	p_H.	Estimated electrometrically.
1	0.2032	4.09	0.204
2	0.2060	4.19	0.209
3	0.1990	4.04	0.198

Assay of Cinchona.—Having shown the use of this method in evaluating weighed samples of crude alkaloids, the method was applied directly to the assay of a sample of red cinchona bark. The following procedure was employed:

The Pharmacopœia process is followed through the point of decanting 160 cc. of the ethereal solvent. This, then, is evaporated to dryness. The residue is treated

once with alcohol and again evaporated to dryness. The alkaloids are then softened by means of neutral ether and dissolved in 9.06 cc. of 0.1*N* hydrochloric acid. The ether is evaporated, the solution made up to 100 cc. and the p_H determined.

It so happened that this sample of cinchona ran higher than 7 per cent. To make the method applicable to this sample of drug 100 cc. of ethereal solvent was employed for the electrometric assay. This represented 2.5 Gm. of drug, the percentage thus obtained from the graph which is based upon 4 Gm. of drug was multiplied by 1.6 to obtain the actual percentage. Sixty cc. of the liquid was subjected to the shaking out process and the alkaloids determined gravimetrically. Table V records the results of these determinations.

TABLE V.

No.	p_H.	Percentage from graph.	Calculated percentage electrometric.	Percentage gravimetric.
1	4.11	5.10	8.16	8.12
2	4.01	4.93	7.90	8.25
3	3.98	4.87	7.80	7.52
4	3.68	4.30	6.90	6.70
5	3.91	4.72	7.55	7.55
6	3.85	4.62	7.40	7.31
7	3.98	4.87	7.80	7.88
8	3.83	4.60	7.36	7.38
9	3.98	4.87	7.80	8.05
10	3.62	4.20	6.72	6.80
11	3.90	4.70	7.53	8.25
12	4.02	4.94	7.91	7.74
			Av. 7.57 per cent	Av. 7.63 per cent

The probable error of a single determination was determined by the formula:

$$r = \pm 0.6745\sqrt{\frac{\Sigma(v^2)}{n-1}}.$$

The probable error of the average of all the determinations was calculated by the formula:

$$R = \pm 0.6745\sqrt{\frac{\Sigma(v^2)}{n(n-1)}}.$$

In these formulas v is the deviation from the mean and n the number of determinations. For the electrometric measurements $r = \pm 0.28$ and $R = \pm 0.08$. For the gravimetric determinations $r = \pm 0.38$ and $R = \pm 0.11$.

Although the means of this series of determinations are practically identical, the probable error of the series by the gravimetric method exceeds the probable error of the electrometric determinations.

Assay of Other Samples of Cinchona.—Other samples of cinchona bark were assayed potentiometrically and gravimetrically. The following results were obtained as given in Table VI.

Equation for Graph.—An examination of the graph reveals that from 6.5 per cent the curve is a straight line. From this point to 3.0 per cent covering practically the range of assay of most samples of cinchona the equation for the curve was calculated. The method of averages was employed using the equation

TABLE VI.

Sample.		Percentage alkaloid gravimetric.	p_H.	Percentage alkaloid potentiometric.
A		5.67	4.40	5.65
		6.04	4.64	6.10
		5.61	4.40	5.65
		lost	4.31	5.53
		5.60	4.38	5.60
		5.53	4.28	5.47
B		5.40	4.26	5.42
		5.67	4.35	5.55
		5.67	4.29	5.48
C		4.50	3.60	4.15
		4.69	3.67	4.27
		4.52	3.65	4.25
			3.75	4.40
			3.66	4.26
			3.74	4.38
D	Very inferior drug	3.24	2.88	3.10
	reported only 3.00	3.31	2.90	3.15
	per cent by analyst	lost	2.94	3.20
E		4.94	4.04	4.97
		4.77	3.96	4.83
		4.78	4.01	4.92
		4.91	4.04	4.97
		4.92	4.05	4.98
		4.94	4.04	4.97

$$y = a + bx.$$

The equation which fits the curve from 6.5 per cent to 3 per cent is

$$\text{Per cent} = 1.7\ p_H - 1.9.$$

Using the procedure of analysis described in this paper this equation makes it unnecessary for the operator to construct his own graph.

CONCLUSION.

A potentiometric method of evaluating the alkaloidal content of cinchona has been devised, which makes the shaking out process unnecessary. It is sufficiently accurate for a determination of this type.

BIBLIOGRAPHY.

(1) R. Dubreuil, *Bull. sci. pharmacol.*, T35 (1928), 635.
(2) W. J. McGill, JOUR. A. PH. A., 11 (1922), 1003.
(3) W. J. McGill and L. R. Wagener, *Ibid.*, 12 (1923), 853.
(4) W. J. McGill and Faulkner, *Ibid.*, 11 (1922), 1003.
(5) L. Maricq, *J. pharm. Belg.*, 11 (1929), 517.
(6) J. C. Krantz, Jr., *Ibid.*, 14 (1925), 294.
(7) Wilson, *Ind. Eng. Chem.*, 17 (1925), 74.
(8) I. M. Kolthoff, JOUR. A. PH. A., 14 (1925), 294.
(9) Popoff and McHenry, *Ibid.*, 14 (1925), 273.

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