Hence it was cohobated, five liters being collected and, upon a second cohobation, 100 cc. This last distillate was opaque and a thin film of oil separated, too small, however, for examination. As already pointed out this distillate had an odor similar to that of the distillate obtained upon hydrolysis of the glucoside.

It is hoped that this preliminary investigation of the seeds of *Bixa orellana* may be repeated with much larger quantities of material.

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ELECTROMETRIC ASSAY METHODS FOR CRUDE DRUGS.*

BY WILLIAM J. MCGILL AND PAUL ELLIS FAULKNER.

One of the most fruitful developments of the electrolytic dissociation theory announced by Arrhenius in 1887 has been the quantitative conception of acidity and alkalinity. Previously, solutions were termed acid, alkaline or neutral, and no attempt was made to interpret different degrees of acidity or alkalinity, although Pasteur had early recognized the effects of these on the growth of microörganisms.

The term "hydrogen-ion concentration" has been used to designate degree of acidity or alkalinity. Since the product of the hydrogen and hydroxyl ions in any aqueous solution must always equal 1.10^{-14} a definite concentration of H-ion is always present, whether the solution be alkaline, acid or neutral, and it is therefore proper to speak of the H-ion concentration of a neutral or alkaline solution as well as of an acid solution. A further development in nomenclature has been the use of the term "p_H value" in place of the more cumbersome "H-ion concentration." The p_H value corresponds to log $1/(H^+)$ and the p_H values for different H-ion concentrations may be calculated from this equation.

The working out of methods for the determination of H-ion concentration has been largely done by Sörenson and his assistants at Copenhagen. Michaelis, in 1914, published his monograph, "Die Wasserstoffionenkonzentration," which summarized the entire subject up to that date. Since that time countless journal articles have appeared dealing with new methods of, and new applications for, the determination of H-ion.

So far, the development of this field has been due in great measure to the desire of workers in the biological sciences for a better knowledge of the effects of

* Scientific Section, A. Ph. A., Cleveland meeting, 1922.

H-ion concentrations upon various life processes. Although a few general articles have been published in pharmaceutical journals, and one short contribution on colorimetric determination of the H-ion concentration afforded by some alkaloidal salts, little experimental work seems to have been stimulated in the field of pharmacy along these lines. From the very nature of the substances with which the pharmaceutical worker has to deal, it would appear that the application of these ideas might prove of value in a number of problems. The deterioration of liquid preparations such as those of digitalis, cocaine, and a number of other easily hydrolyzed substances could be traced by the use of H-ion methods.

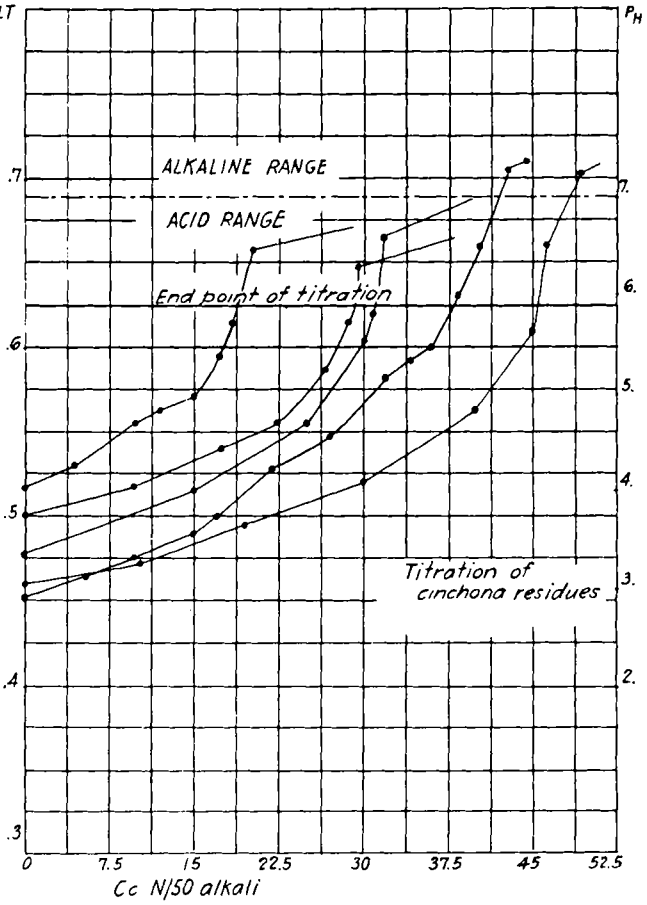
The concentration of H-ion may be determined either electrometrically or colorimetrically. Colorimetric standards are of course based on the hydrogen electrode, and electrometric methods are to be preferred because of their greater accuracy and freedom from interference by colored solutions.

In the assaying of alkaloidal drugs, the conditions seem to be nearly ideal for the use of electrometric titration methods. We are dealing with relatively large masses of inert material from which we endeavor to extract small quantities of active substance in as pure a condition as possible and most of the difficulties involve this effort to obtain a pure alkaloidal residue which may be either weighed or titrated. If some procedure can be utilized in which the usual tedious process of purification of the alkaloidal residue can be avoided, and at the same time equally accurate or even more accurate results can be obtained, a considerable saving of time could be made. Again in the determination of alkaloids by acidimetric titration, we are usually dealing with weak bases which we titrate either directly or indirectly with strong acids. In either case, the factors influencing the end-point are the same, and in practically all cases this comes in an acid solution, but the H-ion concentration of the solution at the correct point for each alkaloid varies widely so that for absolutely accurate results, the indicator must be selected with this fact in mind, and, further, the titration should be carried to a very definite shade of color. Electrometric methods afford the opportunity of avoiding both the long-drawn-out purification and the necessity of choosing the proper indicator and the correct shade of color for the end-point.

The apparatus we have employed is not of a complicated nature. The essentials are a good galvanometer, and potentiometer, besides a standard cell, regulating resistances, and a source of E. M. F., preferably an ordinary dry cell. These, with the hydrogen and calomel electrodes, electric stirrer, burettes, and a 400-cc beaker as a container for the solution, are all that is necessary. The hydrogen used is from a compressed tank supply, and for greater accuracy can be washed through alkaline permanganate, and alkaline pyrogallol solutions. The hydrogen electrodes used are of the Hildebrand type and normal calomel electrodes are employed. The galvanometer, potentiometer, standard cell, source of E. M. F. and regulating resistances may be obtained assembled in one unit in a Leeds & Northrup portable H-ion outfit, and we have found that this assembly is quite accurate enough for alkaloidal titrations. There are many modifications possible, already suggested in the literature or which will suggest themselves to anyone engaged in this work. For instance, it is possible to avoid the use of a potentiometer, the most expensive part of the apparatus, by constructing half-cells generating an E. M. F. equal to that produced in the solution at the finish of the titration, and

determining the null-point by means of an electrometer or galvanometer. Directions for the preparation of half-elements giving different voltages will be found in recent journal articles.

The greater accuracy obtainable by electrometric methods would not alone compensate for the additional equipment and knowledge of the subject involved. Such methods must further prove a saving of time and labor, and this can be accomplished only by shortening the purification process. Impurities to be excluded in an electrometric titration are only those substances which might conceivably exert a buffer action on the solution or cause a marked change in the p_H value of the solution from that which would ordinarily be assumed for the correct end-point. It is hardly possible to foretell the effect of various plant constituents which might be extracted as impurities, and the degree of purification necessary before a residue can be titrated successfully electrometrically has been worked out entirely experimentally.



THE ELECTROMETRIC ASSAY OF CINCHONA.

Because of the relatively large content of alkaloid, cinchona requires a larger number of extractions in the shaking-out process and consequently seemed to be an advantageous beginning for our experiments. Besides this, the titration of the residue always presents difficulties, even when a comparatively colorless residue is obtained, because no matter what indicator we employ the color change is not usually well defined, and the determination is therefore made gravimetrically.

As previously stated, we can titrate electrometrically to any desired H-ion concentration, and the first consideration in the method is to determine the proper end-point for our titration. There are several ways in which this can be done.

In a titration of a cinchona residue indirectly using standard solutions of NaOH and HCl, the solution at the exact end-point would contain the hydrochlorides of the cinchona bases, and sodium chloride, together with the dissociation products

of these substances. The small amount of sodium chloride formed in the neutralization would have little effect on the large volume of solution employed, so that we have only to consider the salts of the alkaloids. Obviously, the p_H value of such a solution should equal that of an aqueous solution of the pure alkaloidal hydrochlorides, of approximately the same dilution, and by determining the p_H value of this latter solution, we know the correct end-point for a cinchona residue titration.

Other methods depend upon plotting the voltage or p_H value against the alkali added, in the case of an indirect titration. If we carry out an electrotitration of a strong acid and a strong base, the curve resulting has a sharp break near the end-point, giving a very steep curve. But in the direct or indirect titration of alkaloids, the break while marked is not so steep, and in order to select the true end-point, it is customary to take the mid-point of the steeper part of the curve and use it as the end-point. In cases where the curve shows no pronounced break, plotting the increase of E. M. F. over the quantity of alkali necessary to produce that addition against the total E. M. F. gives a steep curve whose peak represents the correct end-point.

DETERMINATION OF THE p_H VALUE OF THE HYDROCHLORIDES.

A sample of quinine hydrochloride was carefully purified and various dilutions made up, using boiled, distilled water.

Dilution.	Voltage with N/calomel electrode.	p_H value.
$N/10$	0.628	5.96
$N/50$	0.629	5.98
$N/100$	0.629	5.98
$N/500$	0.631	6.01

It appears that the degree of dilution has little effect on the p_H value, which is to be expected.

A sample of cinchonine hydrochloride purified and dissolved in boiled distilled water gave:

Dilution.	Voltage with N/calomel electrode.	p_H value.
$N/50$	0.614	5.71
$N/500$	0.617	5.77

Since most of the alkaloid content of the samples of cinchona used consists of quinine, it was decided that the fixing of a voltage of 0.625 corresponding to a p_H value of 5.95 with the normal calomel electrode as the end-point would involve only a very slight error.

PREPARATION OF THE SAMPLE.

It was found that a slight modification of the U. S. P. method for the separation of the alkaloid from the crude drug gave a residue sufficiently pure for an electrometric determination.

A sample of five Gm. of the powdered bark is treated with 200 cc of chloroform and after 10–15 minutes' standing 5 cc of ammonia water added and the drug allowed to stand 8–10 hours, shaken 1 hour on a mechanical shaker, and 160 cc of the chloroform solution filtered through cotton into a 250-cc Soxhlet flask. The chloroform is evaporated on the water-bath, the residue which remains ranging all the way from a white crust of the alkaloid to a brown varnish-like residue, depending on the extractive in the drug sample.

TITRATION.

The residue is softened by the addition of 5 cc of neutral alcohol, 15 cc of $N/10$ hydrochloric acid added, and solution effected as far as possible. The liquid is then washed into a 400-cc beaker with several portions of boiled distilled water until the total volume is 300–350 cc.

The titration is carried out just as any electro-titration, either to the definite p_H value already determined, or by plotting the values obtained according to either of the methods outlined. With some experience, a titration can be made in ten minutes. Either $N/10$ or $N/50$ alkali can be used, the latter seeming not markedly more accurate.

COMPARISON OF RESULTS.

Assays were carried out by the above-mentioned methods and the results compared with those obtained in the U. S. P. assay.

Sample.	Electrometric method.	U. S. P. method.	Sample.	Electrometric method.	U. S. P. method.
G	5.33	5.71	F	5.21	..
	5.44	5.68		5.96	6.23
	5.45	5.84		5.89	6.37
	5.32	5.75		5.84	6.18
	5.41	..		..	..
	5.52	..		..	..
	5.50	..		..	..
	6.17	..		..	..

In order to obtain closer basis for comparison the U. S. P. method was carried out exactly, using double the quantities of maceration fluid and of crude drug, 160 cc of the maceration liquid being poured off and assayed by the U. S. P. method of extraction and subsequent weighing, and 160 cc being evaporated directly and assayed according to our method. The results follow:

Sample.	Electrometric method.	U. S. P. method.
A 1	4.14	4.42
2	4.06	4.59
3	4.21	4.47
B 1	6.27	6.44
2	6.09	6.51
3	6.38	6.59
C 1	5.16	5.07
2	5.27	5.42
3	5.09	5.51

In a number of cases the residue in the U. S. P. assay was also titrated, using brom-cresol purple as indicator, but in only a few samples was the end-point satisfactory so that the results could be termed definite.

Curves are given for a number of these titrations although most of the results were calculated on the basis of titrating to a voltage of 0.625 as the end-point.

It will be seen that methods such as the above save a large amount of time and our results, in this case, indicate that they are just as accurate or even more so than the colorimetric method. A number of other drugs have been experimented upon and these results will be discussed in later papers.

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