A PRACTICAL, ACCURATE AND INEXPENSIVE APPARATUS FOR HYDROGEN-ION DETERMINATIONS.*

BY H. K. MULFORD AND F. R. GREENBAUM.

Within the last few years potentiometric hydrogen-ion measurements have been so simplified as to be practical for many industrial uses. More and more the importance of the hydrogen ion is realized, particularly in the fields of pharmaceutical, biological and medicinal preparations. It is desirable to obtain pharmaceuticals, medicinal preparations and biologics at neutral or as nearly neutral as is possible. The colorimetric method, because of its simplicity and the general impression that elaborate and expensive purification of chemicals and complicated calculations are required for the potentiometric method, is still used by many laboratories. It is now possible for an average operator to read over a set of directions, make up a calomel cell from commercially purified chemicals and determine a hydrogen-ion measurement to a high degree of precision in a remarkably short time.

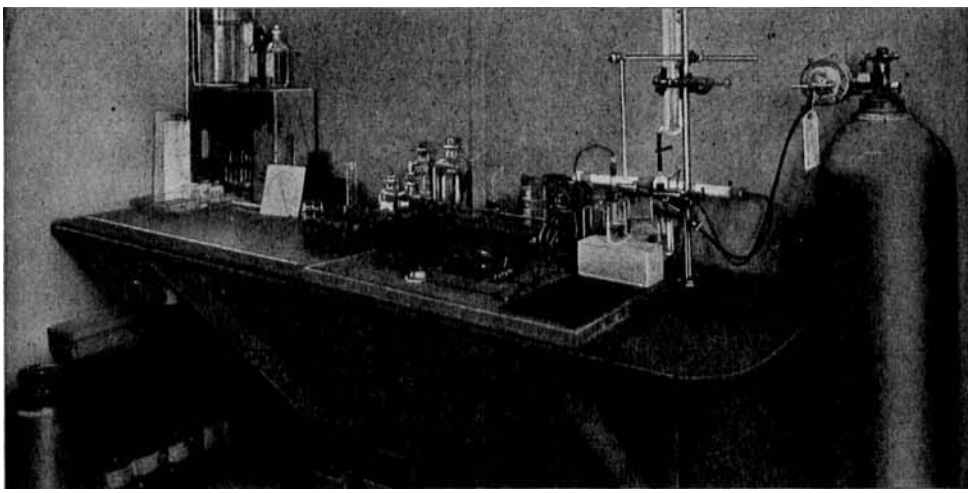

Fig. 1.—Potentiometer for hydrogen-ion determinations.

The apparatus we are using, is comparatively inexpensive, is accurate in its readings and is well suited for research and clinical laboratories and physicians.

Pharmaceutical, medicinal and biological preparations often have a p_H outside the quinhydrone range, particularly above 9, at which range the quinhydrone system is unreliable. Solutions having a p_H of 10 or above 10 are best measured with the hydrogen electrode.

In order to decrease the expense of the hydrogen-electrode apparatus, we have made a hydrogen-ion apparatus,[1] which has been found to be very satisfactory and obtains readings accurate to 0.01. Of the many potentiometric arrangements we use the Hildebrand apparatus, as it appears to be a simple method and at the same time quite accurate.

* Scientific Section, A. PH. A., Portland meeting, 1928.

[1] We have used part of the setting up as suggested by Dr. Gordon Saxon.

The following diagram shows the Hildebrand arrangement: Hildebrand uses an immersion electrode, the successful operation of which depends upon a vigorous stream of hydrogen from the hydrogen tank, bubbling through the solution about the electrode. In the Hildebrand arrangement the hydrogen electrode and the calomel electrode are used as indicated in the diagram. The hydrogen electrode has the advantage of being used, where colorimetric tests fail or are entirely impossible, and it can be connected with an automatically recording electrical apparatus as supplied by Leeds & Northrup Co., of Philadelphia.

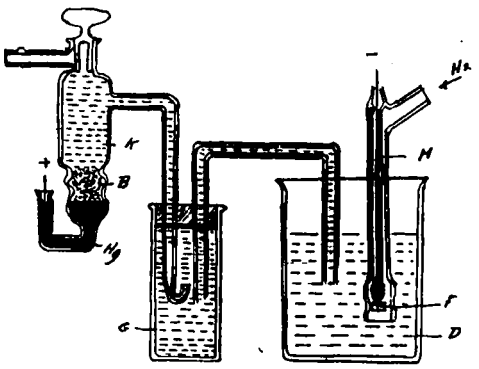

Fig. 2.—Liquid junction potentials—"salt bridge." *Hg*—Mercury (especially prepared). *B*—Calomel. *K*—Saturated KCl. *G*—Connecting vessel. *D*—Liquid of unknown p_H value. *H*—Hydrogen electrode. *F*—Platinum contact.

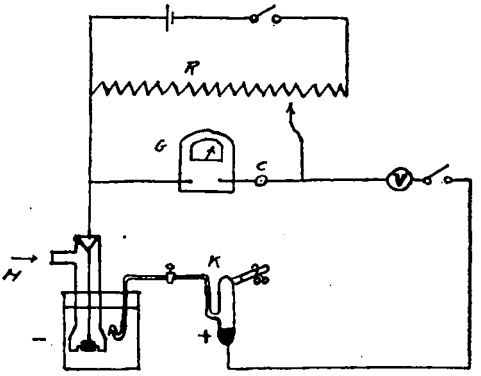

Fig. 3.—Diagram of the Hildebrand apparatus. *R*—Rheostat. *G*—Portable galvanometer. *V*—Voltmeter. *H*—Hydrogen electrode. *K*—Calomel electrode containing sat. KCl. *C*—Contact button.

In order to connect this Hildebrand apparatus we purchased the following articles: one resistance coil, 2 dry cells, 1 voltmeter, 1 galvanometer, 1 hydrogen electrode, 1 calomel electrode, 1 hydrogen tank, one hydrogen-reduction valve, 2 knife switches, 1 bell button and wire. We then mounted the resistance coil, the galvanometer, the voltmeter, with the knife switches and the bell button on a bakelite board, in the arrangement as shown by the above diagram, and connected the positive terminal with the hydrogen electrode and the negative pole with the calomel electrode.

For the preparation of the calomel electrode it was necessary to secure specially prepared mercury, specially prepared calomel and a saturated solution of C. P. potassium chloride. Mercury is added to the cell, until the tip of the platinum wire is well covered. About 7 Gm. of calomel is added and the cell is filled with a saturated solution of potassium chloride, shaken for several minutes and connected with a supply vessel, containing saturated potassium chloride. The pinch clamp of the supply vessel is loosened and the calomel electrode is flushed with saturated KCl, care being taken that no air bubble is formed; all air bubbles must be driven out. The cell is then ready for use. The cell should be flushed between every two or three measurements and also after the measurements are completed, in order to prevent contamination. The next diagram shows the hydrogen electrode and the calomel electrode with the connecting bridge. The hydrogen electrode (point type) must be freshly coated

with platinum before use. They are first cleaned with filter paper, electrolyzed in concentrated hydrochloric acid, washed in water, and dipped in a 3% platinum chloride solution. The current from a two dry-cell battery is allowed to flow through for 1 minute. The electrode is now coated with platinum black and is then electrolyzed in a 10% sulphuric acid, to give it a coating of hydrogen in status nascendi. The calomel electrode and the hydrogen electrode so prepared are now inserted into the liquid, the p_H of which is to be determined, and a vigorous current of hydrogen is passed through, at the rate of about 2–3 bubbles a second. The resistance plug on the resistance coil is moved until the galvanometer no longer shows a deflection. The reading on the voltmeter can now be taken and by means of the hydrogen-ion calculator is transformed into p_H reading. It is most desirable at the beginning and at the end of each series of determinations to check the apparatus with a standard buffer solution of known p_H value. In this way any defect can easily be determined; the main defect is usually the poisoning of the platinum tip of the hydrogen electrode. This apparatus will give accurate p_H readings up to the second decimal point and the cost involved will approximate $75.00.

Instead of using a hydrogen tank, the hydrogen may be prepared chemically in Kipp's apparatus, using C. P. zinc and sulphuric acid; the so-prepared hydrogen being passed through 3% potassium permanganate and through 10% sodium hydroxide solution and finally dried over calcium chloride before entering the hydrogen electrode.

We have determined the p_H of a large number of biological and pharmaceutical preparations and have obtained satisfactory results with this "home made" apparatus.

The following table gives an account of some of our results obtained:

TABLE SHOWING HYDROGEN-ION DETERMINATIONS CARRIED OUT WITH THE ABOVE DESCRIBED HILDEBRAND APPARATUS.

Name of preparation.	Lot no.	Preparation no.	Remarks.	p_H.
Sodium iodide and salicylate of soda with colchicine	8074	61	A sterile	8.20
Sodium salicylate	8076	162	A sterile	8.00
Sodium salicylate	8076	162	B non-sterile	7.35
Iron cacodylate	8075	110	B non-sterile	0.60
Sodium cacodylate	8071	13	A sterile	8.20
Liquor pituitary and epinephrine	8061	143	A sterile	1.95
Anterior pituitary	8046	152	A sterile	3.85
Sodium salicylate	8044	41	A sterile	7.60
Iron citrate compound	8045	7	A sterile	7.93
Sodium iodide	8052	65	A sterile	1.95
Sodium iodide	8052	65	A sterile	1.95
Sodium salicylate	8044	41	B non-sterile	7.20
Iron cacodylate	8055	92	A sterile	1.28
Iron cacodylate	8055	92	B non-sterile	1.74
Iron ammonium citrate comp.	8045	7	B non-sterile	below 0
Sodium citrate	8040	160	A sterile	14.00
Physiological salt solution	8041	100	A sterile	6.60
Physiological salt solution	8041	100	B non-sterile	7.25
Dextrose 50%	8042	175	A sterile	6.60
Dextrose 50%	8042	175	B non-sterile	6.90

Glycerophosphate comp.	8038	134	A sterile	6.85
Glycerophosphate comp.	8038	134	B non-sterile	7.00
Procaine solution	8039	104	A sterile	4.95
Procaine solution	8039	104	B non-sterile	6.45
Iron ammonium citrate	8035	88	A sterile	below 0
Iron ammonium citrate	8035	88	B non-sterile	below 0
Iron cacodylate	8036	54	A sterile	0.55
Iron cacodylate	8036	54	B non-sterile	0.25
Iron cacodylate	5000	54	A sterile	5.30
Iron cacodylate	5001	54	A sterile	1.60
Iron cacodylate	8028	54	A sterile	2.75
Iron cacodylate	8028	54	B non-sterile	1.95
Morphine sulphate	8023	114	A sterile	5.15
Sodium cacodylate	8020	12	A sterile	8.40
Sodium cacodylate	8020	12	B non-sterile	8.40
Sodium iodide and salicylates with colchicine	8019	103	A sterile	8.72
Sodium iodide and salicylates with colchicine	8019	103	B non-sterile	8.72
Glycerophosphates compound	8010	86	A sterile	6.70
Glycerophosphates compound	8010	86	B non-sterile	5.85
Iron cacodylate 2% solution	8018	54	A sterile	3.12
Iron cacodylate 2% solution	8018	54	B non-sterile	2.10
Mercurochrome 1% solution	6346	5	Old ampul	5.90
Mercurochrome 1% solution	6346	5	June 15, 1927	7.22
Mercurochrome 1% solution	6346	5	October 31, 1927	7.85
Mercurochrome 1% solution	6346	5	June 12, 1928	8.55
Procaine and epinephrine	8017	132	A sterile	2.55
Procaine and epinephrine	8017	132	B non-sterile	2.65
Magnesium sulphate	8004	25	A sterile	5.85
Magnesium sulphate	8004	25	B non-sterile	6.85

We have obtained, as can be seen from this table, accurate readings with this apparatus and have been able to cover the range from 0–14.

It is interesting to note the difference of the p_H in sterile and non-sterile preparations. Of course it depends on the chemical nature of the preparation as to how the sterilization will influence the p_H. In some cases the p_H will be slightly influenced by sterilization, such is the case in procaine and epinephrine solution, sodium cacodylate, sodium iodide and many others.

Other preparations, however, will change the p_H on the acid side by sterilization, such is the case in physiologic salt solution, dextrose, glycerophosphates compound, procaine, magnesium sulphate and many others and finally some preparations on sterilization show an increase in the p_H, which means they become less acid, as in iron cacodylate.

The hydrogen-electrode apparatus, however, is unsuitable for determining the hydrogen-ion concentration of solutions containing certain metals. Among these metals might be mentioned copper, arsenic, bismuth, antimony and mercury; of course gold and other noble metals will have the same action. The reason for these unsatisfactory results on some organic metal solutions is probably due to the reduction of the electrode by the metal ion—the metal ions below hydrogen in the electro-chemical series reducing the hydrogen electrode.

For measurements of this type of solution the quinhydrone electrode may be employed. In order to use our above-described apparatus it was only necessary to obtain one gold electrode and some quinhydrone, both rather inexpensive items.

Then by replacing the hydrogen electrode with the gold electrode and adding a small amount of quinhydrone to the metal organic solution the p_H can be readily determined with the same Hildebrand arrangement and in this way gives a combined hydrogen and quinhydrone apparatus.

From the foregoing we hope we have made it quite clear that at comparatively little expense an accurate hydrogen-ion apparatus may be built, entirely suitable for the needs of pharmaceutical, medicinal and biological chemists.

RESEARCH LABORATORIES,
THE NATIONAL DRUG CO., PHILADELPHIA, PA.

STUDIES IN BIOASSAYS—"THE TINCTURE OF STROPHANTHUS."*[1]

BY CHARLES W. EDMUNDS, M.D.,[2] HAROLD W. LOVELL[3] AND SPENCER BRADEN.[3]

The U. S. Pharmacopœia directs that Tincture of Strophanthus shall be of such a strength that 0.0006 cc. of the tincture shall be equivalent in strength to 0.0005 mg. of ouabain when tested upon frogs by the official method. Burn and Trevan have pointed out that this is equivalent to saying that 1 cc. of the tincture must equal in activity 8.33 mg. of ouabain. These workers assayed six tinctures of strophanthus by the cat method, and found all of them to be considerably weaker than the U. S. P. requirement, averaging an ouabain equivalent of about 3.5 mg. instead of 8.33 mg.

Other writers at an earlier date have reported upon the activity of tinctures of strophanthus as they appear upon the market, but some of these papers reported conditions existing in this country before a biological standard was required, or in other instances conditions existing in localities to-day where such standards are still not demanded. For this reason it will hardly be necessary to discuss their findings at length. The Burn-Trevan paper will be discussed later, as it is an important criticism of the U. S. P. standard, which perhaps should be lowered in the next revision, if it is, as stated, unreasonably high. It was in an effort to shed further light upon the question that we examined a number of tinctures of strophanthus which were obtained in original, unopened bottles direct from the manufacturers. We employed the same methods and standards which we had used in our study of ouabain and strophanthin as outlined in the section of this paper which appeared in an earlier number of THIS JOURNAL.

The bioassay methods employed were as follows: the various frog methods, including the official one-hour method; the intravenous method; the four-hour method and in one or two instances the minimal lethal dose method. We used also the cat-assay method and finally the colorimetric method. In addition to using ouabain as a standard we employed also a special tincture prepared from Strophanthus Kombé seeds which we obtained through the courtesy of Dr. Paul S. Pittenger. These seeds which were obtained direct from a "Kombé" district in Africa and identified as being of that variety, were ground and made into a tincture according

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* From the Pharmacological Laboratory of the University of Michigan.

[1] Supported in part by a grant from the Board of Trustees of the U. S. Pharmacopœia.

[2] Professor of Materia Medica and Therapeutics, University of Michigan.

[3] U. S. P. Fellows in Pharmacology in the University of Michigan.