Gas electrode for general use / by G.S. Walpole.

No. 69

GAS ELECTRODE FOR GENERAL USE

BY

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XLI. GAS-ELECTRODE FOR GENERAL USE.

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The standard forms of hydrogen-electrode, such as those used by Dolezalek [1899], Wilsmore [1900] and Bjerrum [1910] and, for fluids containing carbon dioxide, Hasselbalch [1910], must remain indispensable when physical measurements of the highest order of accuracy are undertaken.

With these, after the expenditure of considerable time and care, and with continued vigilance over every part of the electrical apparatus, consecutive results concordant to about 0·05 millivolt can sometimes be obtained. But, for these results to have any meaning beyond the nearest millivolt or two, it is essential that the diffusion potential difference of the cell should be accurately known in every determination: and as this is recognised, by Cumming and others whose investigations continue to throw light on this difficult subject, to be almost impossible, it follows that, as far as absolute $H^{\cdot}$ ion determinations are concerned, attention should be focussed more particularly on diffusion potential errors (in view of their greater magnitude) than on those found at the electrode itself.

In general laboratory practice, therefore, especially when dealing with protein-containing materials, which may or may not be free from carbon dioxide, the most suitable electrode is not one which, when coupled with an ideal electrical apparatus, will give results of this high order of accuracy, regardless of the expenditure of time and material. Rather it is one which, without exaggerating appreciably the diffusion potential error, will give, under ordinary working conditions and in a few minutes, results correct to 1 millivolt or so on one or two cc. of fluid without loss or contamination.

Anticipating a long series of $H^{\cdot}$ ion determinations on protein solutions, some of them containing carbon dioxide, I endeavoured to devise such an electrode. The arrangement subsequently adopted and the experiments made to discover its imperfections are described below. Only one form of

apparatus has been used for all cases. When dealing with a solution containing carbon dioxide or other dissolved gases a slight modification of technique is all that is necessary.

Apparatus.

The modifications of existing apparatus which I have employed consist of

(1) the electrode vessel,
(2) the filling apparatus,
(3) the support in the constant temperature bath.

The electrode vessel (*ABD*, Fig. 1) is somewhat more complicated than the simple V shape generally employed, but this is amply compensated for by the simplicity of the other parts of the apparatus and the many conveniences accompanying its use. I have used throughout the platinum point advocated by Michaelis, just making contact with the surface of the fluid. The platinum point is mounted at the end of a glass tube *A*. The protruding end is blackened in the usual manner. The other end makes contact with a small globule of mercury placed inside the tube.

The lower end of *A* may be ground to fit the outer tube *B* or else a joint may be made by means of a rubber stopper as in the diagram. Attached to *B* at the side is a very fine bore capillary tube carrying a tap *D*, and at the lower end is a second capillary tube not quite so fine, of about 1 mm. bore. At the lower end of this may be fitted a very small glass stopper, but it is not necessary and has certain disadvantages.

The filling apparatus used with all these electrode vessels is depicted together with other apparatus in Fig. 2. It consists of a three-way piece *F* connecting a 5 cc. all-glass syringe *G*, well lubricated with vaseline, a short piece of fine-bore stout rubber tubing *H* and a glass tube carrying a glass stopcock which is connected with the hydrogen supply.

As *support* for the electrode vessels in the constant temperature bath, while taking potentiometer readings, I have used a glass trough *K* (Fig. 1) in which the electrode vessels stand side by side in a suitable connecting solution. For single electrode vessels a large test tube serves excellently. The other half-electrode employed, a tube from which leads into the glass trough, is also immersed in the water-bath.

I have used principally the calomel-saturated potassium chloride half-electrode recently described by Michaelis and Davidhoff [1912] and a standard calomel N/10 KCl half-electrode. The connecting solutions were saturated potassium chloride solution, saturated ammonium nitrate [Cumming, 1907],

and solutions of potassium chloride 1·75 N and 3·5 N respectively, employed for the extrapolation method of Bjerrum.

In some experiments on diffusion potential two gas electrodes of the vertical type were stood side by side in the trough containing connecting solution.

Experiments with solutions free from carbon dioxide and other dissolved gases.

Technique. The electrode vessel is first of all connected by the capillary side tube *D* (Fig. 1) to the rubber tube of the filling apparatus (shown at *GFH*, Fig. 2), and hydrogen passed for a few seconds, both glass taps being open. Then, by alternately drawing out the piston with the tap on the electrode vessel shut, and pushing it home with the tap open, the last traces of air are expelled from the dead space of the syringe and the T-piece. The lower end of the electrode vessel is now brought under the surface of a sample of the fluid to be examined and the glass tap on the T-piece shut. By pulling out the plunger of the syringe the fluid is drawn up until its surface is just at the point where the platinum point is sealed to the glass. Slight movements of the plunger in and out now cause the liquid by rising and falling to wash the platinum point well without wetting the tube *A* (Fig. 1) supporting it. Finally the height of the liquid is adjusted till the point just touches the surface, the glass tap *D* on the side capillary closed, and the vessel containing the remainder of the sample of fluid taken away.

The electrode vessel is now disconnected from the filling apparatus, and the lower capillary wiped dry with a piece of clean filter-paper and placed in the glass trough in a constant temperature bath. There is sufficient connecting solution in the trough to cover the electrode vessel above the rubber stopper. The two copper wires from the potentiometer are now led, one into the calomel half-electrode, and the other into the tube *A* and the reading is taken from which the value of P_H^+ (the negative exponent of the $H^{\cdot}$ ion concentration) may be calculated directly.

In order to be certain that the platinum electrode is working properly and that equilibrium is established instantaneously, a few minutes at least may be allowed to elapse before a second reading is taken. Meanwhile the filling apparatus can be used to fill another electrode vessel with another sample, and when this is introduced into the trough, the copper wire can be changed over from the first electrode vessel and a reading of that taken. While the equilibrium of the second electrode is being checked, the first can

be refilled. It is removed, dipped into a large beaker of distilled water, and dried externally with a clean towel or filter paper and connected again to the filling apparatus.

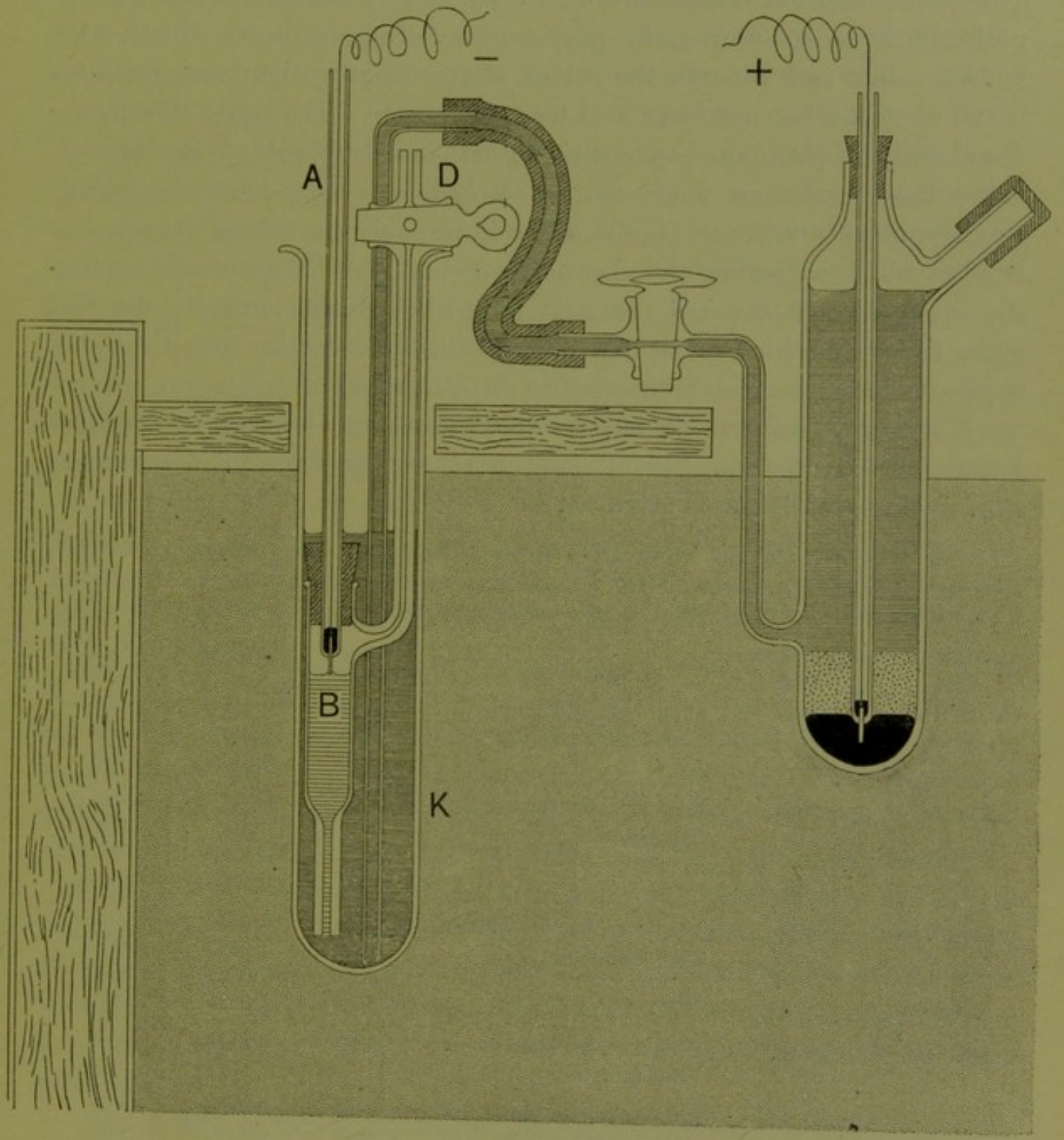

Fig. 1.

Opening the tap *D* (Fig. 1) and pushing home the syringe, the electrode vessel is emptied, and the tap on the filling apparatus opened for a few seconds to fill it with fresh hydrogen. The next sample having been brought underneath the electrode vessel, the tap on the three-way piece is closed,

liquid drawn up by the syringe, expelled so as to get rid of traces of the previous sample, and then the vessel filled with a fresh sample as before.

If an electrode is properly blacked, and in good working order, it should give the equilibrium value immediately and scarcely change afterwards—two millivolts at most—during the next ten or twenty minutes. Diffusion of hydrogen into and through the rubber stopper causes slight inconvenience if the electrode has not been used for a day or two. The liquid rises in the vessel and the platinum becomes immersed beyond the point. Equilibrium under these conditions would not be reached for a long time. To rectify this, the glass tube *A* can be slid upwards through the rubber stopper. It is advisable when setting the electrode aside, to push the glass tube holding the electrode well through the cork, fill with hydrogen, suck up distilled water till the platinum is immersed and after disconnection from the filling apparatus leave the vessel immersed in distilled water.

Washing out of electrode vessel. One rinsing only between two samples has been found sufficient. Several experiments were performed to demonstrate this. One set of figures is given below.

Temp. 18°. Bar. 750 mm. Half-electrode sat. KCl-calomel. Connecting fluid sat. KCl. Calculated P_H^+ value for 0·0993 N HCl (dissociation 91·6 %) = 1·041. Calculated P_H^+ value 0·1 N NaOH (14·14 − 1·075) = 13·065. Sample used was known not to be absolutely free from CO_2.

Solution used	Volume used	Remarks	Time of observation	Potential π	$P_H^+ = \frac{\pi - 0{\cdot}251}{0{\cdot}0577}$
0·0993 N HCl	2 cc.	Apparatus at commencement clean and dry	8.45	0·3110	1·039
			8.50	0·3110	1·039
			9.10	0·3113	1·045
,,	4 cc.	Used 2 cc. for rinsing out last sample	1.45	0·3113	1·045
			2.00	0·3115	1·048
			2.15	0·3120	1·057
			2.45	0·3120	1·057
0·1 N NaOH	4 cc.	Used 2 cc. for rinsing out last sample	2.50	1·0020	13·015
			2.55	1·0022	13·019
			3.15	1·0022	13·019
			3.50	1·0030	13·032
0·0993 HCl	4 cc.	Used 2 cc. for rinsing out last sample	4.19	0·3115	1·048
,,	circ. 20 cc.	Rinsed out many times and filled side capillary, see page 425. Passed bubble for 5 mins.	4.45	0·3105	1·031
			4.55	0·3110	1·039
			5.00	0·3110	1·039
			next morning		
			8.5	0·3108	1·036

Temperature. Although most of these determinations have been made at 18° exactly, it is unnecessary to pay too great attention to this point as long as the temperature of the calomel-saturated KCl half-electrode and the gas

electrode are the same in any one determination. Calculation from the constants given by Michaelis for this combination demonstrates that the error in measurement of the value of P_H^+ per 1° is 0·007 for a neutral solution the H· ion concentration of which has no temperature coefficient. The error with a N/10 KCl-calomel electrode is about three times this. Approximate determinations with the saturated KCl half-electrode may quite well be made on the open bench without a constant temperature bath.

When a N/10 KCl-calomel half-electrode or a N/10 HCl-hydrogen half-electrode are used in combination with the gas electrode, more careful temperature adjustment is necessary. As a direct result of the diminutive size of the electrode vessel, and the small quantity of fluid used therein, the time taken to arrive at temperature equilibrium is very short.

Quantity of material used. The size of electrode vessel found most convenient for general use has the following principal dimensions; length of tube *B* (Fig. 1) 75 mm., maximum external diameter 10·5 mm., length of lower capillary 25 mm. The capacity when full is 1·5 cc., so that a determination can be performed easily on 2 cc. of the material if the electrode vessel be clean and dry to start with. When the electrode vessel is not cleaned and dried between two determinations, 4 cc. are required—2 cc. to rinse out the vessel and 2 cc. from which the electrode vessel is filled.

If only smaller quantities of material are available, a smaller electrode vessel may be used. Quite good results were obtained by one holding 0·3 cc. This is not necessary, however, for the quantity of material available may be drawn up into a vessel of the most convenient size and then saturated potassium chloride drawn up afterwards until contact is made with the platinum point. Mixing of the two fluids only takes place very slowly by diffusion unless the density of the fluid examined approximates to that of saturated KCl. In those cases when it is known that the H· ion concentration does not change by such treatment the sample may be diluted with pure water [cf. Michaelis and Davidhoff, 1912]. This is the substitution of an indirect method for a direct one and so an additional possibility of error is introduced.

The extent of the contamination of the fluid during a determination is very small since the boundary surface between it and the saturated potassium chloride solution is only that of a section of the capillary tube. When a very small glass stopper is fitted at the bottom of the capillary, possibility of contamination is still further removed. It is usually found that enough potassium chloride diffuses round the stopper to diminish the resistance sufficiently for a reading, not always very sharp, to be taken.

In many cases, therefore, where a small quantity of liquid only is available, it is possible to determine its reaction electrometrically and recover it with very little contamination, dilution, or loss, for further experiments. Usually a moderate economy of material is advantageous and, on this and other grounds, the use of the vertical electrode filled by suction has been found by me much more convenient than the V-type for general work. Frothing fluids do not exhibit their disagreeable characteristic, as hydrogen is not bubbled through them, but they are forced up by external atmospheric pressure. There is no need, therefore, to make an indefinite contact with the froth. The abolition of tapes or wool threads soaked in potassium chloride solution was found a great convenience.

Diffusion Potential. In the electrolytic cells of which one half-electrode is hydrogen in contact with an aqueous solution there are really four differences of potential involved. There are the two electrode potentials proper and two differences of potential where the electrode solutions come into contact with the connecting solution. The latter differences of potential are for the most part dependent upon the natures of the electrode solutions and of the connecting solution, but the time the solutions have been in contact is a concomitant factor. Cumming and Gilchrist [1913] have recently investigated this quantitatively and find that this "time change" is more marked with capillaries than open tubes. They, therefore, recommend that capillaries should be avoided in the construction of an electrolytic cell; so also should membranes, cotton wool plugs and, presumably, tapes.

In the vertical electrode vessel filled by suction, the surface between the solution examined and the connecting fluid is in a capillary or at the end of one. It may readily be made to occur at the wider part of the tube (p. 415, paragraph 4). I have made a number of careful measurements with this cell to determine how far variations in potential difference can be traced to boundary changes, and to what extent these alter when the surface between the electrode solution and the connecting solution is in the broader part of the tube instead of at the end of the capillary.

Using N HCl and N/10 HCl as electrode solutions connected to a saturated KCl-calomel half-electrode by saturated KCl solution, I could not detect with certainty any difference in the time change over half an hour in each case—first with boundary at end of capillary, second with boundary in broader part of the tube. Over longer periods, differences in the "time changes" could probably be detected, but do not rightly enter into consideration in the ordinary use of the electrode.

I have sometimes used saturated ammonium nitrate [Cumming, 1907]

instead of saturated potassium chloride as connecting fluid when examining acid solutions. With the N/10 KCl-calomel half-electrode N and N/10 HCl gave the potentials 0·3390 and 0·3974 respectively; calculated values 0·3407, 0·3977. With the saturated KCl-calomel electrode, however, the results were about 18 millivolts too low, the contact potential between saturated potassium chloride solution and saturated ammonium nitrate being of that order.

Comparison between the results obtained with the vertical and the V*-electrode vessels.*

Both electrode vessels were fitted with rubber stoppers through which passed the glass tube in the end of which the blackened platinum point was mounted. When necessary this was adjusted so as just to touch the surface of the liquid. Temp. 18°. Connecting solution saturated KCl solution.

Date	Solution	Half-electrode	Potential readings: V-electrode vessel	Potential readings: Vertical electrode vessel	Previous results
18 April	0·01 Na_2CO_3 Aq.	Calomel. Saturated KCl	0·8770	0·8780	—
11 April	Sodium Citrate (Sörensen)	,, ,, ,,	0·6240	0·6235	0·6238 (Sörensen)
6 May	Standard Acetate solution (Michaelis)	,, ,, ,,	0·5165	0·5170	0·5175 (Michaelis)
7 May	0·1 N HCl	,, ,, ,,	0·3122	0·3125	—
8 May	0·1 N HCl	,, ,, ,,	0·3122	0·3125	—
15 May	N HCl	,, 0·1 N KCl	0·3435	0·3425	—
15 May	N HCl	,, Saturated KCl	0·2558	0·2560	—

Electrometric Titration.

In acidimetry and its various applications the addition of a standard alkaline solution is continued until the titrated fluid has a certain $H^{\cdot}$ concentration previously agreed upon. This is called the "end point." Generally the "titre" or amount of standard alkali which must be added to a certain amount of the fluid to be titrated in order to arrive at the "end point" is all the information required. The observer, with a proper understanding of the nature of the reaction, knows the correct "end point" for the object he has in view, and chooses an indicator which will tell him by its colour change when the "end point" is reached.

For instance, in the titration of an acetic acid solution it is desired to add standard NaOH solution until all the acetic acid is converted to sodium acetate. The hydrogen-ion concentration of sodium acetate solution is about $P_H^+ = 7{\cdot}5$ to $9{\cdot}5$ so that phenolphthalein or another indicator exhibiting its colour change over this range of reaction is employed.

Again, in the Kjeldhal titration N/10 NaOH is added to a very dilute solution of sulphuric acid containing some ammonium sulphate. The "end point" is reached when all the sulphuric acid is converted to ammonium sulphate and sodium sulphate. Ammonium sulphate is faintly acid so that a trace of soda in excess will bring the mixture nearly neutral. Hence an indicator is chosen which is very sensitive at the neutral point—methyl orange, alizarin, rosolic acid have all been recommended. Such simple titration processes depend for their accuracy and reliability on a sudden change taking place in the reaction of the titrated fluid at some point during the addition of the alkaline solution, and the correct choice of an indicator which demonstrates this change. When dealing with very feebly dissociated acids and bases, of which proteins and some of their products of hydrolysis are excellent examples, such sudden changes of reaction do not occur. The titration process becomes more difficult. Moreover at any time it gives no information as to the rate of change of concentration of H˙ ions taking place progressively step by step as the alkaline solution is added. The measurement of this is important, and its neglect is frequently the outcome of the fact that it is a laborious process. To do it colorimetrically would be tedious and, in small quantities of fluid, impossible. Electrometrically with an electrode vessel of the standard type and a tape it would be impracticable, since loss and contamination of the fluid by potassium chloride would inevitably occur. With a vertical electrode vessel filled by suction these measurements of change of H˙ ion concentration during the titration process have been found quite practicable. There is no loss, practically no contamination by potassium chloride, and each determination of H˙ ion concentration during the titration process takes about two minutes.

Apparatus. Fig. 2 is a scale drawing of the apparatus used for titrating 10 cc. quantities. A small beaker contains the fluid to be titrated and dipping into it is a small electrode vessel (*B*) of capacity 3 cc. and a capillary tube filled with saturated KCl solution, connected to the saturated KCl-calomel electrode. Passage of liquid bodily along the capillary is prevented by the insertion of a long tightly packed plug of cotton wool, and if necessary a clip on the rubber tube connecting it to the half-electrode. The conductivity of the potassium chloride is so good that readings may still be taken with the clip on and sometimes, if the tap on the half-electrode be not vaselined, with this tap shut. A standard burette is clamped above the beaker. Two wires from the potentiometer lead to the half element and the electrode vessel respectively.

Technique. The electrode vessel is filled with hydrogen, and, while the

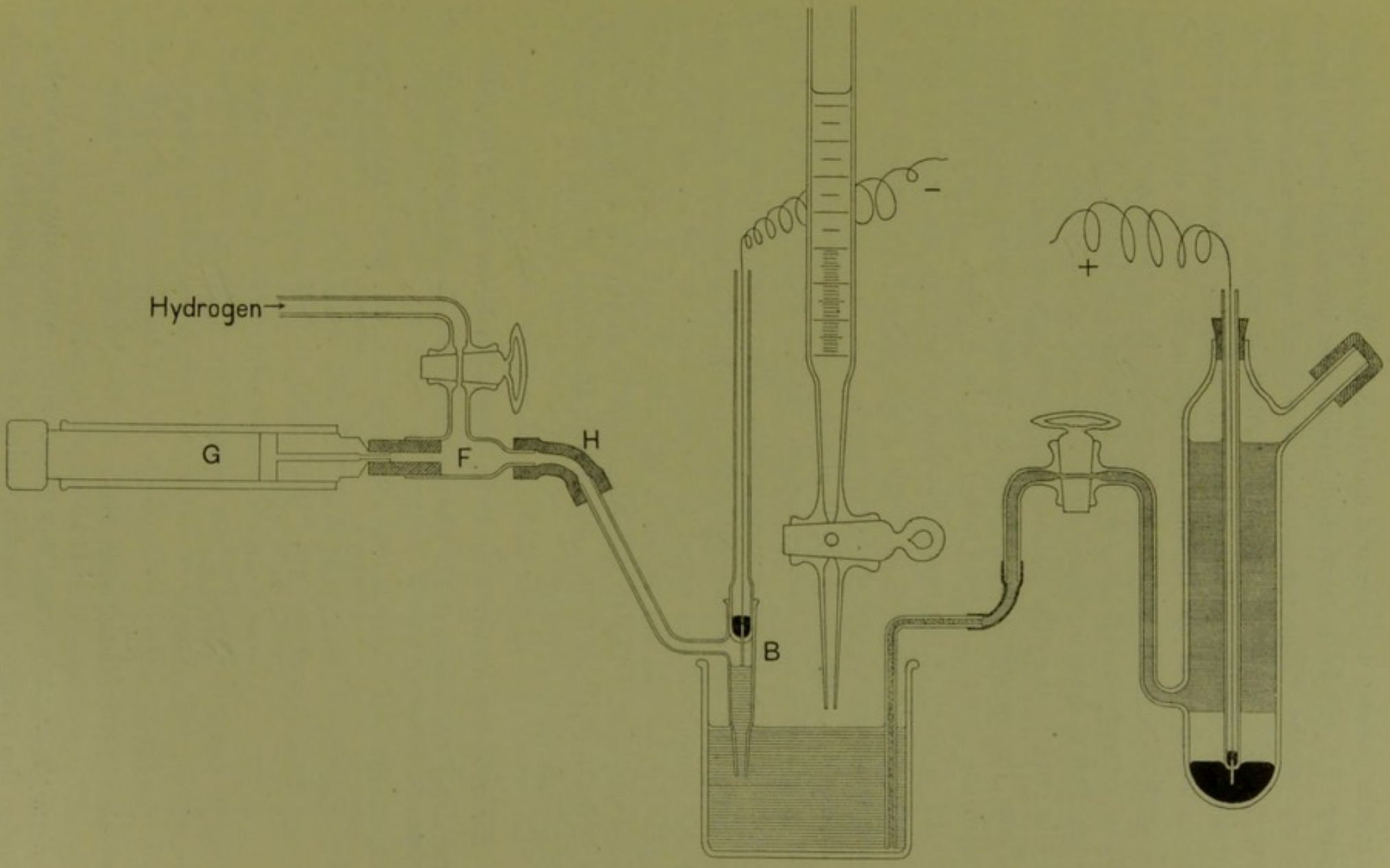

Fig. 2.

gas is still passing, its lower end brought below the surface of the measured quantity of liquid to be titrated which has been introduced into the beaker. The tap on the filling apparatus is turned off and by withdrawing the plunger, liquid is sucked up until contact is made. A reading of the potential indicates the reaction of the original liquid. The plunger is pushed home, a few drops of the titrating solution in the burette are run in, and by imparting a rotary motion to the beaker its contents are mixed until uniform throughout, and the reaction determined as before. In practice it is found best to draw the liquid up into the side tube and push it out several times in order to rinse the platinum point well between each reading.

From the results a curve may be plotted showing the relationship between the quantity of fluid added and the reaction. If any doubt exists as to the accuracy of the results obtained in this way, one or two points on the curve can be checked by single additions of N/10 alkali followed by determinations in a larger electrode vessel.

Example 1. 10 cc. of N/10 glycocoll solution containing in 1 litre 7·505 g. glycocoll and 5·85 g. sodium chloride [Sörensen, 1909, 1] were titrated with N/10 HCl electrometrically. Two separate titrations *A* and *B* were performed and subsequently the amount of potassium chloride accidentally introduced was found to be equivalent to 3·3 cc. of decinormal solution in one case and 3·0 cc. in the other, though no clip was used and the tap was open. The 10 determinations in titration *B* took 13 minutes; the 21 determinations in titration *A* took 43 minutes, though no particular attempt was made at speed.

Electrometric titrations of 10 *cc. glycocoll solution with N*/10 *HCl.*

Connecting fluid—saturated KCl solution. Half-electrode—saturated KCl-calomel. No correction for diffusion potential.

Vol. N/10 HCl added	E.M.F. in titration *A*	E.M.F. in titration *B*	Calculated from Sörensen's results
1 cc.	0·446, 0·445, 0·445	0·443	0·446
2	0·426, 0·426, 0·426	0·425	0·426
3	0·413, 0·414, 0·414	0·412	0·413
4	0·404, 0·404	0·402	0·403
5	0·394, 0·394	0·393	0·394
6	0·387, 0·387	0·386	0·386
7	0·381	0·379	0·379
8	0·374	0·373	0·373
9	0·369	0·367	0·367
10	0·365, 0·365, 0·365	0·362	0·362

Example 2. Advantage was taken of the opportunity now afforded of following the changes of reaction during a Sörensen formaldehyde titration.

A 4 % solution of Witte peptone was employed and the titration performed with every care. First colorimetrically, compensating for the colour of the peptone solution by a special tintometer, and then electrometrically.

1. Sörensen titration of 4 % Witte peptone solution colorimetrically.

Stage (1). *The neutralisation of the peptone solution.* Neutral red solution was used as indicator, and as a standard of neutrality a mixture of N/15 phosphate solutions (34·4 cc. acid + 65·6 cc. alkaline). 10 cc. of this solution —its reaction does not change on dilution [Szili, 1904]—to which 1 cc. of neutral red solution was added, were placed in a cylindrical vessel with a flat bottom *D*. A similar vessel surmounting this contained 10 cc. of Witte peptone *B*. Two corresponding vessels, *A* and *C*, contained distilled water, and 10 cc. Witte peptone solution plus 1 cc. neutral red solution respectively. Looking down *AC* the tint appeared redder than that seen looking down *BD*, and successive additions of N/10 NaOH solution were made until 0·5 cc. had been added, and the two columns were seen to match. It was considered that the 10 cc. of Witte peptone solution were neutralised by the addition of 0·5 cc. N/10 NaOH. Unfortunately the colorimetric result is misleading here. The P_H^+ value is 7·66 and not 7·07. The errors in P_H^+ found by Sörensen [1909, 2] in similar determinations with 2 per cent. Witte peptone solution containing 0·1 N NaCl and correcting for the colour of the solution by 2 drops of Bismarck brown and 2 drops of helianthin II were 0·18 and 0·12 in the same direction in two cases. Rosolic acid gave a similar result.

Stage (2). *Titration in presence of formaldehyde.* The problem is to discover how much 0·1 N NaOH must be added to a mixture of 10 cc. of Witte peptone solution and 10 cc. of neutral 40 per cent. formaldehyde solution in order that the resulting mixture should have the reaction $P_H^+ = 8{\cdot}68$ (7 borate + 3 HCl); what further addition must be made to bring P_H^+ value to 8·91 (8 borate + 2 HCl); and what still further addition will bring the value to $P_H^+ = 9{\cdot}09$ (9 borate + 1 HCl).

Using the same apparatus the solutions were arranged as shown on p. 422.

Formaldehyde bleaches slightly the colour of Witte peptone so that its colour must be compensated for by a solution similarly bleached. 1 cc. saturated sodium chloride solution is added to each formaldehyde-peptone mixture to inhibit the formation of polymethylimino-compounds. The same quantity was added to the 20 cc. of Sörensen mixture in *D* in order that the "neutral salt effect" on the phenolphthalein should be of the same order in

A.

10 cc. Witte peptone solution.
10 cc. formaldehyde containing 1·0 cc. of 0·1 % phenolphthalein in 50 % alcohol.
1 cc. saturated NaCl solution.
(4·8 cc. 0·1 N NaOH were added to this tube to make a match.)

B.

10 cc. Witte peptone solution.
10 cc. formaldehyde solution.
1 cc. saturated NaCl solution.

C.

20 cc. distilled water.

D.

20 cc. Sörensen mixture.
(7 borate + 3 HCl.)
1·0 cc. of 0·1 % phenolphthalein in 50 % alcohol.
1 cc. saturated NaCl solution.

A and *D*. The amount of 0·1 N NaOH added to *A* before a match resulted was 4·8 cc. *D* was now removed and replaced by *D'* containing 20 cc. (8 borate + 2 HCl) mixture, 1·0 cc. of 0·1 % phenolphthalein in 50 % alcohol, and 1 cc. saturated sodium chloride solution. A further 0·3 cc. of 0·1 N NaOH was required in *A* to make a match. *D'* was now replaced by *D''* containing 20 cc. (9 borate + 1 HCl) mixture, 1·0 cc. of 0·1 % phenolphthalein in 50 % alcohol, 1 cc. saturated NaCl. A further 0·3 cc. of 0·1 N NaOH was required to make a match. Hence by a colorimetric method it has been found that the following mixtures have the corresponding H· ion concentrations given in the table:

P_H^+	Composition of mixture
7·07	10 cc. Witte peptone solution + 0·5 cc. 0·1 N NaOH
8·68	,, ,, ,, + 10 cc. neutral 40 % formaldehyde sol. + 4·8 0·1 N NaOH
8·91	,, ,, ,, + ,, ,, ,, + 5·1 ,,
9·09	,, ,, ,, + ,, ,, ,, + 5·4 ,,

By a simple artifice the formation of polymethylimino-compounds in the above titrations may be prevented without the addition of sodium chloride solution. If, before adding the formaldehyde to the peptone solution some 0·1 N NaOH be added, then no cloudiness or precipitate forms. Repeating the above titrations in this manner and adding the solutions in the order named, the following results were obtained.

P_H^+							
8·68	10 cc. Witte peptone soln.	+	4·0 cc. 0·1 N NaOH	+	10 cc. neutral 40 % formaldehyde + 0·80 cc. 0·1 N NaOH
8·91	,, ,, ,,	+	,, ,,	+	10 cc. neutral 40 % formaldehyde + 1·10 cc. 0·1 N NaOH
9·09	,, ,, ,,	+	,, ,,	+	10 cc. neutral 40 % formaldehyde + 1·40 cc. 0·1 N NaOH

In Fig. 3, the relation between the compositions of these mixtures and their P_H^+ values is represented diagrammatically.

The points obtained using phenolphthalein are marked +. The neutral red point is marked #.

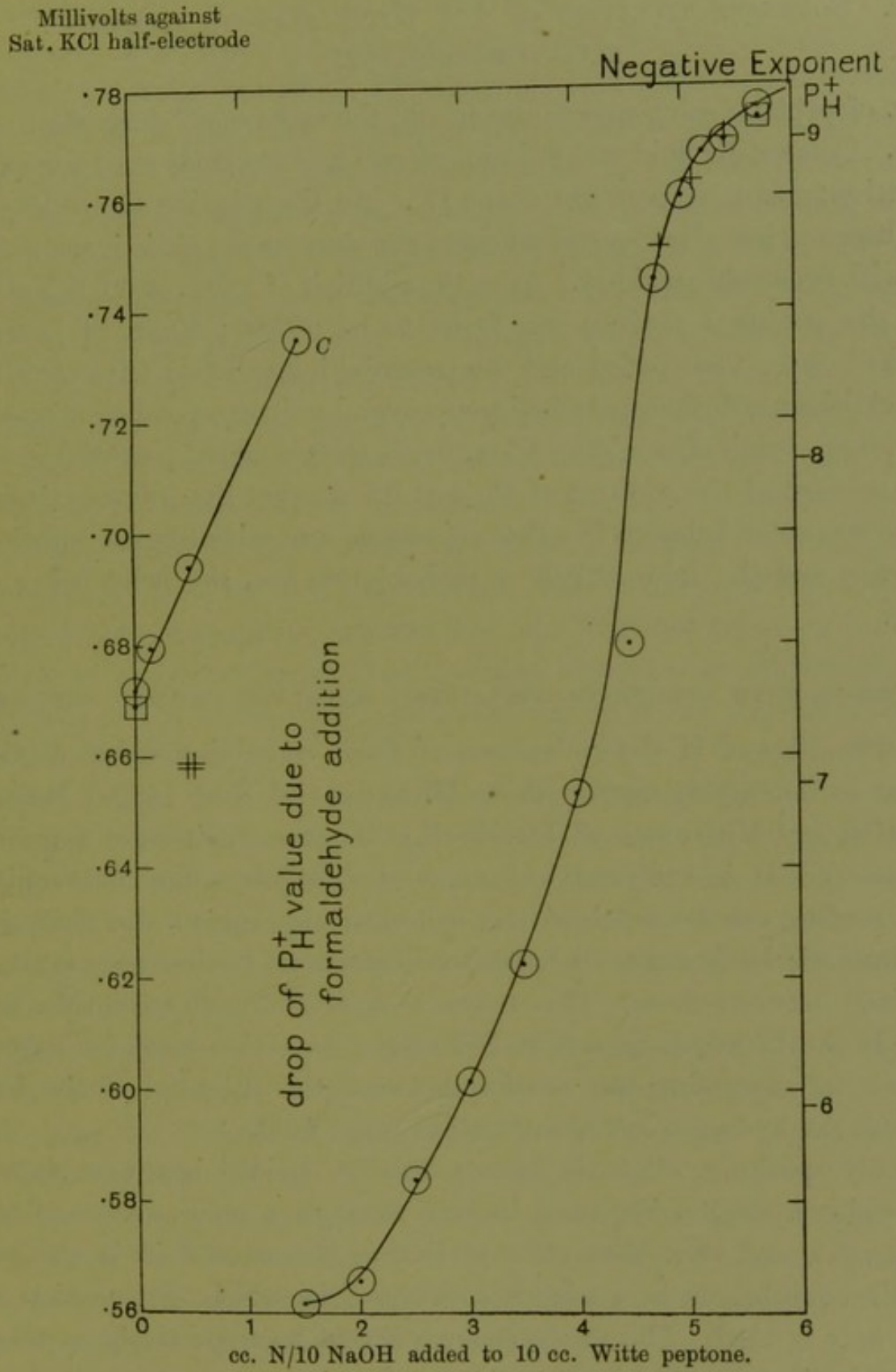

Fig. 3. Sörensen formaldehyde titration of 4 % Witte peptone performed electrometrically.

Note.—At *C* 10 cc. neutral formalin was added.

⊙ are points obtained in the titration process—electrode containing 0·3 cc.

□ are points obtained with large electrodes in separate determinations.

\+ are points obtained colorimetrically using phenolphthalein.

is point obtained colorimetrically using neutral red.

2. Sörensen titration of 4% Witte peptone solution electrometrically.

10 cc. of peptone solution were taken and the same small electrode vessel as before. Connecting fluid and fluid in calomel half-electrode were saturated KCl solution; room temperature about 17°. As the alkaline formaldehyde solution becomes less alkaline on prolonged exposure to the air it is necessary to work with reasonable rapidity. After the addition of 1·5 cc. of 0·1 N NaOH solution the potential reading was found to be 0·7350; 10 cc. of neutral formaldehyde were then added and the potential immediately fell to 0·5615. Further additions of decinormal alkali were made—and corresponding potential readings taken—until after adding 4·5 cc. in all, the change of potential was so great, on account of the addition of the last 0·5 cc., that the ordinary rinsing out of the apparatus between two determinations was evidently not sufficient for this case, and the value 0·6800 is probably too low, the point being off the curve.

Experiments with solutions containing dissolved carbon dioxide.

The difficulties of H· determinations of fluids containing carbon dioxide have been so thoroughly dealt with by Michaelis and Rona [1909], Hasselbalch [1910], and Michaelis and Davidhoff [1912] that their repetition here is unnecessary. It is sufficient to remark that before a final unchanging potential reading can be obtained the hydrogen atmosphere, the fluid, and the platinum electrode must all be in equilibrium, no further gas exchange taking place between them. This refers to hydrogen, carbon dioxide, and oxygen. If the hydrogen be not at 760 mm. a correction must be applied to the formula expressing the relation between the potential of the half element and the hydrogen ion concentration of the fluid.

Generally speaking, electrode vessels may be divided into two classes, those in which hydrogen is passed in turn through a small portion of the fluid examined, and then through a portion of the same fluid in the cell itself until equilibrium is attained, and those in which one portion of hydrogen only is used. The methods are referred to respectively as those of the "moving" and "still" hydrogen atmosphere.

In electrode vessels in which a "still" atmosphere is employed it may be considered that there are three degrees of accuracy with which the H· concentration of a fluid containing carbon dioxide may be determined.

Firstly, the bubble of hydrogen may be brought to the surface of the fluid examined, the blackened platinum point saturated with hydrogen

adjusted so that it is just in contact with this surface, and the potential measured. The form of electrode used may be the V-electrode or the vertical electrode described. The potential will not represent accurately the reaction of the fluid. Owing to the diffusion of carbon dioxide from the fluid into the hydrogen the surface layers will rapidly become more alkaline than the solution was originally. The potential reading will therefore be high, falling slowly as equilibrium is established and only reaching a constant value some hours afterwards when that end has been attained.

Secondly, the small bubble of hydrogen may be passed backwards and forwards through the fluid in the cell a few hundred times before a reading is taken. By this means equilibrium is established, and it will be found that the value is constant and nearly correct. The electrode used may be a V-electrode manipulated in accordance with the instructions given by Michaelis [1912], or the vertical type filled by suction, or the Hasselbalch electrode. It will be seen that, though equilibrium is established between the hydrogen atmosphere and the fluid, the fluid has given up some carbon dioxide to the hydrogen and has therefore become more alkaline than it was originally and it is not the $H^{\cdot}$ ion concentration of the original solution that has been measured.

This brings us to the third step where after equilibrium is reached the hydrogen bubble is retained, but the fluid in the cell replaced by a fresh volume with which the hydrogen is again brought into equilibrium. This process may of course be repeated until a definite final potential reading is observed which will then represent the true reaction of the fluid containing carbon dioxide.

The only electrode vessel described which permits of this is the Hasselbalch electrode vessel. Michaelis used it to control the results obtained by him using the V-electrode and passing the bubble up and down many times to obtain equilibrium—the second case above.

Since finding that the same thing can be done quite simply with the same electrode vessel as that described for gas-free solutions, I have made a number of determinations of the reactions of carbonate solutions by its means and have obtained consistent results.

Technique. If the electrode vessel be tipped sideways when filling, the liquid drawn up may be made to enter the side capillary leaving a bubble of hydrogen. After closing the tap the apparatus may be taken in the hand and by a slight movement at the wrist the bubble made to pass from one end of the vessel to the other as many times as are necessary to obtain equilibrium.

Bringing the vessel into a vertical position again the lower end is dipped below the surface of the fluid in the beaker, and the glass tube moved up or down through the rubber stopper until the platinum point just touches the surface of the column of fluid standing up in the electrode vessel. This may now be wiped dry externally, placed in the trough, and a reading of the potential taken. The value obtained corresponds exactly to that obtained by the V-tube used in the manner advocated by Michaelis. When small quantities only of carbon dioxide are present, this value will be very nearly correct. It may be checked by attaching a rubber tube to the tube at *D* (Fig. 1), introducing the lower end of the vessel in a sloping position into the beaker again, aspirating at *D*, and opening the tap gently. In this way fresh solution is drawn into *B* in the place of the old solution which passes out through *D* without disturbing the hydrogen bubble. This fresh quantity of solution is now brought into equilibrium with the bubble of hydrogen as before. The process may be repeated indefinitely and the result obtained is of the third order of accuracy—previously only obtained by the Hasselbalch electrode. The pattern of electrode vessel having a ground glass joint cannot be used for these operations as the height of the platinum point in the vessel is not then adjustable. It is essential that the platinum point shall only just touch the surface of the fluid when a reading is to be taken, otherwise equilibrium between the electrode and the solution, instead of taking only a minute or two, may take hours. In these cases, where the investigated liquid contains carbon dioxide, with experiments lasting over a number of hours, the rubber joint has a further disadvantage over and above that already mentioned. Carbon dioxide, like hydrogen, permeates rubber, and though the rubber joint is immersed in potassium chloride solution, transpiration of gases through the rubber is not prevented, and this slow transpiration is detrimental to accurate work when experiments last several hours.

Carbonate solutions. In order to check the results obtained when using the vertical electrode filled by suction for fluids containing carbon dioxide, I have determined the hydrogen ion concentration of mixtures of a sodium carbonate solution and dilute hydrochloric acid. For each determination 12·5 cc. of 0·2 N sodium carbonate were taken and diluted to nearly 100 cc. Then a measured quantity of 0·1 N hydrochloric acid was added and the volume made to 100 cc. exactly. No barometric correction has been applied to the determinations for diminished hydrogen pressure due to the carbon dioxide present. Neither have any steps been taken beyond the use of saturated potassium chloride as connecting solution to correct for diffusion potential. The results are plotted on a curve (Fig. 4). Abscissae represent

cc. of 0·1 N HCl taken: while ordinates are proportionate to the P_H^+ values less a constant. It will be seen that a mixture of 12·5 cc. of 0·2 N sodium carbonate + 12·5 cc. 0·1 N HCl diluted to 100 cc. corresponds to a solution of 0·0125 molecular $NaHCO_3$ which is also 0·0125 N with respect to sodium chloride. At this point the potential is 0·7200 against the calomel saturated-KCl half-electrode corresponding to $P_H^+ = 8{\cdot}13$. At this reaction phenolphthalein gives a pale pink colour, thus confirming the propriety of the analytical device of titrating carbonates in the presence of caustic alkali using phenolphthalein and methyl orange.

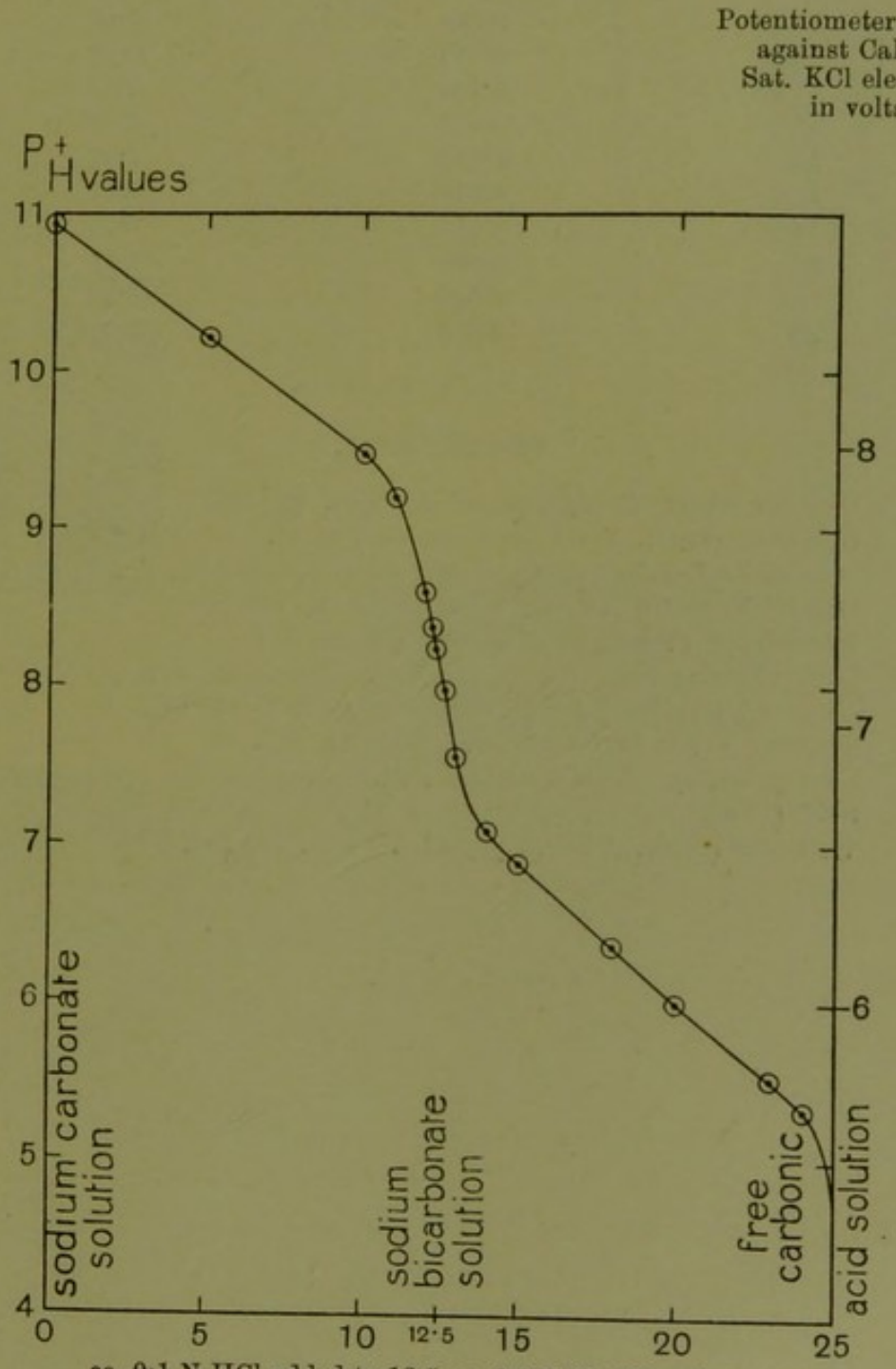

Fig. 4.

Mixtures of 12·5 *cc.* 0·2 *N* Na_2CO_3 *and varying quantities of* 0·1 *N HCl. diluted to* 250 cc.

Connecting fluid sat. KCl. Half-electrode calomel-sat. KCl.

Vol. of 0·1 N HCl	Potential π	$P_H^+ = \frac{\pi - 0{\cdot}251}{0{\cdot}0577}$
0	0·883	10·95
5	0·841	10·22
10	0·798	9·48
11	0·782	9·20
12	0·755	8·73
12·3	0·738	8·44
12·5	0·720	8·13
12·7	0·701	7·80
13	0·684	7·50
14	0·652	6·95
15	0·638	6·71
18	0·618	6·36
20	0·598	6·01
23	0·570	5·53
24	0·558	5·32

REFERENCES.

Bjerrum (1910), *Zeitsch. physikal. Chem.* **73**, 724.
Cumming (1907), *Trans. Faraday Soc.* **11**, 1.
—— and Gilchrist (1913), *Trans. Faraday Society.*
Dolezalek (1899), *Zeitsch. Elektrochem.* **5**, 533.
Hasselbalch (1910), *Biochem. Zeitsch.* **30**, 317.
Michaelis and Rona (1909), *Biochem. Zeitsch.* **18**, 317.
—— and Davidhoff (1912), *Biochem. Zeitsch.* **46**, 131.
Sörensen (1909, 1), *Biochem. Zeitsch.* **21**, 167.
—— (1909, 2), *Biochem. Zeitsch.* **21**, 245.
Szili (1904), *Zeitsch. Elektrochem.* 113.
Wilsmore (1900), *Zeitsch. physikal. Chem.* **35**, 291.