Investigation on the Biilmann's Quinhydrone Electrode.

IV. Comparative Study of Quinhydrone and Hydro-quinhydrone Electrode.

By

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[July 20, 1929]

This paper deals with the further investigation on the BIILMANN's quinhydrone electrode in regard to the following points:

1. comparative merits of BIILMANN's quinhydrone and hydro-quinhydrone electrode in various fluids;

2. test on the simplified, saturated KCl-calomel comparing with the standard N/10 KCl-calomel electrode especially as to their practical use.

Recently some investigators have shown that the quinhydrone electrode is less reliable in some cases than the hydro-quinhydrone electrode. For instance, GROSSMAN[1] found in connection with the blood and body-fluids that the hydro-quinhydrone electrode gives steadier and more accurate results than the quinhydrone electrode. BIILMANN and KATAGIRI[2] showed that the presence of glucose and alcohol in the test fluid renders the quinhydrone electrode unsteady. SHIKATA and TACHI[3] also demonstrated that the presence of ammonium ion makes the quinhydrone electrode less effective and unsteady.

Since these factors are met often in course of the microbiological investigation, it is very important to know as to the behavior of these different electrodes so that proper choice as well as the correct use can be made.

Hydro-quinhydrone Electrode.

The hydro-quinhydrone electrode was devised by BIILMANN[4] and is very similar to the quinhydrone electrode in its manipulation except the mixture of hydroquinone and quinhydrone (10 : 1) is added to the test fluid and a platinum

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1) GROSSMAN, F., Biochem. Jour., 21, 267, 1927.

2) BIILMANN, E. and H. KATAGIRI, Biochem. Jour., 21, 441, 1927.

3) SHIKATA, M. and I. TACHI, Jour. Biochem. (Japan) 10, 115, 1928.

4) BIILMANN, E., Ann. Chim., 15, 109, 1021; —— and LUND, Ann. Chim., 16, 321, 1921.

wire electrode is completely immersed in the precipitate formed by the excess of the reagent.

The PH value is calculated according to the following formulae:

Formula I. $P_H = 4.85 - \frac{\pi}{0.0577}$, 18° C.

is used, for

$$\text{Hg—HgCl} \;\Big|\; \text{N/10 KCl} \;\Big|\; \text{conc. KCl} \;\Big|\; \begin{matrix}\text{Unknown solution +}\\ \text{Hydro-quinhydrone}\end{matrix} \;\Big|\; \text{Pt (blank)}$$

Formula II. $P_H = 6.37 - \frac{\pi}{0.0577}$, 18° C.

is used, for

$$\text{Hg—HgCl} \;\Big|\; \text{sat. KCl} \;\Big|\; \text{conc. KCl} \;\Big|\; \begin{matrix}\text{Unknown solution +}\\ \text{Hydro-quinhydrone}\end{matrix} \;\Big|\; \text{Pt (blank)}$$

Experimental I.

Comparative Test of Quinhydrone and Hydro-quinhydrone Electrode.

The test was carried out with the following buffer solutions and various nutrient media as noted separately on the following pages.

The hydrogen gas electrode was used as the control with which other two electrodes were compared, and the following apparatus and materials were used:

Potentiometer: K-type, Leeds and Northorup.

Quinhydrone: prepared at the laboratory.

Hydroquinone: Soc. of Chem. Industry in Basle (Switzerland).

For the hydro-quinhydrone electrode, plain platinum wire electrode was used, and the mixture of 1.0 g. of hydroquinone and 0.1 g. of quinhydrone was used per 15 cc. of test solution.

1. SÖRENSEN's Buffer Solution[1].

The results of comparative tests with Sörensen's buffer solution are given in Table I. The determinations were made with N/10 KCl-calomel electrode.

The buffer solution of different PH was made up as follows:

PH 4.70 — 4.98 : M/5 Na_2HPO_4 + M/5 KH_2PO_4.

PH 8.52 — 8.75 : 0.05 M Borax + 0.1 M HCl.

1) SÖRENSEN, S. P. L., Compt. rend. Lab. Carlsberg, 8, 1, 1909.

Table I.

Sörensen's Buffer Solution. (17° C)

Electrode / No.	π			PH			Difference.	
	H_2	Q.	Qh.	H_2	Q.	Qh.	H_2—Q.	H_2—Qh.
1.	0.8403	—0.1381	—0.2070	8.75	8.75	8.47	±0.00	+0.28
2.	0.8279	—0.1249	—0.1988	8.52	8.52	8.32	±0.00	+0.20
3.	0.7805	—0.0773	—0.1510	7.71	7.70	7.47	+0.01	+0.24
4.	0.7616	—0.0595	—0.1382	7.38	7.38	7.26	±0.00	+0.12
5.	0.7292	—0.0263	—0.1130	6.81	6.80	6.81	+0.01	±0.00
6.	0.7077	—0.0060	—0.0920	6.45	6.45	6.45	±0.00	±0.00
7.	0.6741	+0.0278	—0.0578	5.86	5.86	5.86	±0.00	±0.00
8.	0.6237	+0.0789	—0.0070	4.98	4.98	4.98	±0.00	±0.00

Notes.

H_2—hydrogen gas electrode.
Q.—quinhydrone "
Qh.—hydro-quinhydrone " .
(H_2—Q)—difference of PH values obtained by the two electrodes.
(H_2—Qh)— " " " " " " " " " .
These notations will be used through-out this publication.

As the above table indicates that the quinhydrone electrode gave very close results as a whole to those obtained by the hydrogen electrode while the hydro-quinhydrone gave the smaller PH value where the PH value is larger than 7. The similar behavior of hydro-quinhydrone electrode in the alkaline solution was noted by GROSSMAN[1] who proposed the correction equation,

$$\triangle \text{PH} = 0.24 \times 2^{\text{PH}-7} - 0.21, \text{ which requires}$$

further investigation.

2. KOLTHOFF's Buffer solution.[2]

The similar experiment was carried out with KOLTHOFF's buffer solution which was made up as follows:

PH 3.84 — 4.85 : 0.05 M Succinic acid + 0.05 M Borax.
PH 5.77 — 8.57 : 0.1 M KH_2PO_4 + 0.05 M Borax.

The results are given in Table II.

1) GROSSMAN, Loc. Cit.
2) KOLTHOFF, I. M., J. Biol. Chem., 63, 135, 1925.

Table II.

Kolthoff's Buffer Solution. (18° C)

Electrode / No.	π			PH			Difference.	
	H_2	Q.	Qh.	H_2	Q.	Qh.	H_2 – Q.	H_2—Qh,
1.	0.8330	—0.1193	—0.1953	8.57	8.41	8.24	+0.16	+0.33
2.	0.8017	—0.0890	—0.1684	8.02	7.89	7.76	+0.13	+0.26
3.	0.7653	—0.0546	—0.1402	7.36	7.29	7.28	+0.07	+0.08
4.	0.7370	—0.0263	—0.1138	6.86	6.80	6.81	+0.06	+0.05
5.	0.6733	+0.0344	—0.0524	5.77	5.76	5.76	+0.01	+0.01
6.	0.6183	+0.0876	+0.0013	4.84	4.84	4.84	±0.00	±0.00
7.	0.5599	+0.1440	+0.0593	3.84	3.85	3.84	—0.01	±0.00

As Table II indicates, the hydro-quinhydrone electrode gave wider variation in those PHs larger than 7, from the hydrogen electrode than the quinhydrone electrode.

3. CLARK's Buffer Solution.[1]

The CLARK's buffer solution was tried and the following results were obtained:

Table III.

Clark's Buffer solution. (16° C)

Electrode / No.	π			PH			Difference.	
	H_2	Q.	Qh.	H_2	Q.	Qh.	H_2—Q.	H_2—Qh.
1.	0.8378	—0.1340	—0.1880	8.76	8.69	8.13	+0.07	+0.63
2.	0.8151	—0.1112	—0.1715	8.37	8.29	7.85	+0.08	+0.52
3.	0.7870	—0.0876	—0.1516	7.88	7.88	7.50	±0.00	+0.38
4.	0.7445	—0.0433	—0.1242	7.14	7.11	7.02	+0.03	+0.12
5.	0.6776	+0.0232	—0.0648	5.96	5.95	5.98	+0.01	—0.02
6.	0.6271	+0.0727	—0.0150	5.07	5.09	5.12	—0.02	—0.05
7.	0.5666	+0.1317	+0.0480	4.03	4.05	4.03	—0.02	±0.00

As Table III indicates, the hydro-quinhydrone gave lower PHs as PH increases and also irregular results.

With all these buffer solutions, it was clearly demonstrated that both the quinhydrone and hydro-quinhydrone electrode give lower PHs as the PH increases and it is more marked wih the latter.

4. Tests on the Culture Media.

The similar tests as with the buffer solutions were carried out on the culture media, as follows:

1) CLARK. M., The Determination of Hydrogen Ions. 1922.

a) Standard Nutrient Broth.

In order to ascertain the comparative behavior of quinhydrone and hydro-quinhydrone electrode in presence of protein and ammonium ion, the standard nutrient broth was inoculated with B. subtilis, and the PH was determined at different intervals. The amount of ammonia at the time of determination together with the PH are noted in Table IV.

Table IV.

Nutrient Broth. (18° C)

Electrode	Time	Plain broth, NH_3·N (trace)		B. subtilis, 2nd day, NH_3·N (1.31%)		B. subtilis, 5th day, NH_3·N (3.66%)	
	min.	π	PH	π	PH	π	PH
H_2		0.7173	6.57	0.7649	7.40	0.8161	8.28
Q.	0.	—0.0095	6.52	—0.0533	7.27	—0.1071	8.20
	1.	—0.0094	6.51	—0.0526	7.27	—0.1058	8.19
	3.	—0.0091	6.51	—0.0514	7.23	—0.1042	8.15
	5.	—0.0087	6.51	—0.0503	7.23	—0.1031	8.14
	10.	—0.0075	6.49	—0.0481	7.18	—0.1005	8.08
Qh.	0.	—0.0844	6.33	—0.1249	7.02	—0.1607	7.64
	1.	—0.0844	6.33	—0.1249	7.02	—0.1607	7.64
	3.	—0.0844	6.33	—0.1249	7.02	—0.1607	7.64
	5.	—0.0844	6.33	—0.1249	7.02	—0.1607	7.64
	10.	—0.0844	6.33	—0.1249	7.02	—0.1607	7.64

Table IV. indicates that the quinhydrone electrode gave very close results as the H_2-electrode while the much lower results were obtained by the hydro-quinhydrone electrode. It however is true that the PH values obtained by the quinhydrone decreased as the amount of ammonia increased as well as the time elapsed during the determination. On the other hand, the hydro-quinhydrone gave higher PH values and steady readings under the similar conditions, which is in agreement with the observations made by Shikata and Tachi[1]. From these results, it may be stated that the quinhydrone can be used advantageously in case of broth in presence of ammonia providing the determination is carried out quickly viz. within one minute after the quinhydrone is added.

b) Glucose Broth.

To determine the effect of glucose in broth on the electrodes noted previously, the standard broth with various amount of glucose added, was taken and inoculated with B. mycoides, and the determination of PH was carried out at different intervals as noted in Table V:

1) SHIKATA and TACHI, Loc. cit.

Table V.

Glucose Broth. (18° C)

Electrode	Time	0.5 % Glucose Broth				1 % Glucose Broth				2 % Glucose Broth			
		Plain		B. mycoides, 5 days old.		Plain		B. mycoides, 5 days old.		Plain		B. mycoides. 5 days old.	
	min.	π	PH	π	PH	π	PH	π	PH	π	PH	π	PH
H_2		0.7090	6.43	0.6161	4.81	0.7039	6.34	0.6194	4.86	0.7030	6.33	0.6164	4.81
	0	−0.0025	6 40	+0.0835	4.89	+0.0019	6.32	+0.0795	4.98	+0.0023	6.32	+0.0849	4.89
	1.	−0.0017	6·39	+0.0841	4.89	+0.0023	6.32	+0.0805	4.95	+0.0029	6.30	+0.0847	4.88
Q.	3.	−0.0008	6.37	+0.0847	4.88	+0.0032	6.30	+0.0812	4.95	+0.0038	6.28	+0.0850	4.88
	5.	−0.0000	6.35	+0.0852	4.88	+0.0039	6.28	+0.0817	4.93	+0.0046	6.26	+0.0850	4.88
	10.	+0.0017	6.32	+0.0859	4.86	+0.0060	6.25	+0.0828	4.91	+0.0063	6.25	+0.0853	4.88
	0	−0.0840	6.31	−0.0018	4.89	−0.0830	6.29	−0.0015	4.89	−0.0830	6.29	−0.0020	4.89
	1.	−0.0840	6.31	−0.0018	4.89	−0.0830	6.29	−0.0015	4.89	−0.0830	6.29	−0.0020	4.89
Qh.	3.	−0.0840	6.31	−0.0018	4.89	−0.0830	6.29	−0.0015	4.89	−0.0830	6.29	−0.0020	4.89
	5.	−0.0840	6.31	−0.0018	4.89	−0.0830	6.29	−0.0015	4.89	−0.0830	6.29	−0.0020	4.89
	10.	−0.0840	6.31	−0.0018	4.89	−0.0830	6.29	−0.0015	4.89	−0.0830	6.29	−0.0020	4.89

The results seem to indicate that very close results were obtained by all the electrodes. In other words, the presence of glucose has no ill effect on the electrodes used.

c) ASHBY's Mannite Solution.[1]

The similar experiment, as noted previously, was carried out with ASHBY's mannite solution, with and without the inoculation of Azotobactor chroococcum. The results are noted in Table VI:

Table VI.

Ashby's Mannite Solution (1%) 18° C.

Electrobe	Time	Plain mannite Solution		Azot. chroococcum, 14 days old.		Azot. chroococcum, 40 days old.	
	min.	π	PH	π	PH	π	PH
H_2		0.7761	7.59	0.8118	8.21	0.8086	8.16
Q.	0	−0.0563	7.32	−0.0563	7.34	−0.0681	7.54
	1.	−0.0570	7.34	−0.0570	7.34	−0.0684	7.54
	3.	−0.0573	7.34	−0.0570	7.34	−0.0682	7.54
	5.	−0.0574	7.34	−0.0569	7.34	−0.0678	7.52
	10.	−0.0574	7.34	−0.0566	7.34	−0.0672	7.52
Qh.	0	−0.1092	6.74	−0.1195	6.93	−0.1175	6.90
	1.	−0.1092	6.74	−0.1195	6.93	−0.1175	6.90
	3.	−0.1092	6.74	−0.1195	6.93	−0.1175	6.90
	5.	−0.1092	6.74	−0.1195	6.93	−0.1175	6.90
	10.	−0.1092	6.74	−0.1195	6.93	−0.1175	6.90

The close results to those of hydrogen electrode were obtained by the quinhydrone than by the other in the medium without inoculation. There is a wide difference among the results obtained by different electrode where the organism was grown.

d) Cellulose Medium.[2]

The similar experiment was carried out with the cellulose medium which was used for cultivation of B. thermofibrincolus[3], and the results shown in Table VII:

(Table VII. s. P. 262.)

Here, again it is shown that the quinhydrone electrode gave the closer results than the hydro-quinhydrone, and in both cases the PH values were lower even the medium became acid on the third day.

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1) ASHBY, S. E., J. Agr. Science, 2, 38, 1907.

2 & 3) ITANO, A. and S. ARAKAWA, in print.

Table VII.

Cellulose Medium. (18° C)

Electrode.	Time.	Plain Cellulose medium.		B. thermofibrincolus., 3 days old.	
	min.	π	PH	π	PH
H_2		0.7781	7.62	0.6850	6.01
Q.	0.	−0.0511	7.23	+0.0206	5.99
	1.	−0.0511	7.23	+0.0213	5.99
	3.	−0.0510	7.23	+0.0219	5.97
	5.	−0.0507	7.23	+0.0222	5.97
	10.	−0.0502	7.22	+0.0231	5.95
Qh.	0.	−0.1269	7.05	−0.0590	5.88
	1.	−0.1269	7.05	−0.0590	5.88
	3.	−0.1269	7.05	−0.0590	5.88
	5.	−0.1269	7.05	−0.0590	5.88
	10.	−0.1269	7.05	−0.0590	5.88

e) WILLIAM's[1], and Dextrose-tartaric acid[2] Media :

Two media for cultivation of yeasts, namely WILLIAM's and dextrose-tartaric acid were tried, with and without inoculation of Sacch. cerevisiae. The results are noted in Table VIII :

Table VIII.

William's and Dextrose-tartaric acid Media. (18° C)

Electrode	Time.	WILLIAM's medium.				Dextrose-tartaric acid medium.			
		Plain.		Sacch. cerevisiae 8 days old.		Plain.		Sacch. cererisiae 8 days old.	
	min.	π	PH	π	PH	π	PH	π	PH
H_2		0.5971	4.48	0.5267	3.27	0.5287	3.31	0.5194	3.13
Q.	0.	+0.1165	4.33	+0.1805	3.21	+0.1862	3.13	+0.1885	3.08
	1.	+0.1174	4.33	+0.1815	3.20	+0.1871	3.11	+0.1912	3.04
	3.	+0.1183	4.31	+0.1825	3.18	+0.1875	3.11	+0.1924	3.02
	5.	+0.1187	4.29	+0.1832	3.18	+0.1878	3.09	+0.1931	3.01
	0.	+0.1194	4.29	+0.1839	3.16	+0.1880	3.09	+0.1933	3.01
Qh.	0.	+0.0263	4.41	+0.0915	3.26	+0.0880	3.33	+0.1000	3.13
	2.	+0.0263	4.41	+0.0915	3.26	+0.0880	3.33	+0.1000	3.13
	3.	+0.0263	4.41	+0.0915	3.26	+0.0880	3.33	+0.1000	3.13
	4.	+0.0263	4.41	+0.0915	3.26	+0.0880	3.33	+0.1000	3.13
	5.	+0.0263	4.41	+0.0915	3.26	+0.0880	3.33	+0.1000	3 13

1) WILLIAM, R. T., J. Biol. Chem. 42, 259, 1920.

2) STARKEY, R. L. and HENRICI, A. T., Soil Science, 23, 3

In these media, it appears that the hydro-quinhydrone is suited better but the difference is very small in either case.

Discussion on Experimental I:

From these results obtained in the foregoing experiments, it may be stated that the quinhydrone electrode can be used satisfactorily in these media, tried under various conditions providing the readings are taken soon after the reagent is added. On the whole, the quinhydrone gave closer results to those obtained by the H_2-electrode than the hydro-quinhydrone.

Experimental II.

Comparative Test of Simplified Saturated[1] and N/10 Standard Calomel Electrodes.

The accuracy of simplified saturated electrode was tested against in chain with H_2, quinhydrone and hydro-quinhydrone electrode by using three different buffer solutions, namely SÖRENSEN's, KOLTHOFF's, and CLARK's. The results are noted in Table IX:

Table IX.

Comparative tests of Standard N/10 KCl-calomel and Itano's Simplified Saturated KCl-caromel Electrode.

Buffer Solution	H_2			Q.			Qh.		
	N / 10	Saturated	Difference of PH	N / 10	Saturated	Difference of PH	N /10	Saturated	Difference of PH
SÖRENSEN's	8.75	8.82	—0.07	8.75	8.81	—0.06	8.47	8.52	—0.05
	8.52	8.59	—0.07	8.52	8.58	—0.06	8.32	8.36	—0.04
	7.71	7.78	—0.07	7.70	7.75	—0.05	7.47	7.51	—0.04
	7.38	7.45	—0.07	7.38	7.43	—0.05	7.26	7.29	—0.03
	6.81	6.88	—0.07	6.80	6.86	—0.06	6.81	6.85	—0.04
	6.45	6.52	—0.07	6.45	6.50	—0.05	6.45	6.49	—0.04
	5.86	5.91	—0.05	5.86	5.93	—0.07	5.86	5.87	—0.01
	4.98	5.05	—0.07	4.98	5.03	—0.05	4.98	5.00	—0.02
KOLTHOFF's	8.57	8.64	—0.07	8.41	8.47	—0.06	8.24	8.29	—0.05
	8.02	8.09	—0.07	7.89	7.97	—0.08	7.76	7.81	—0.05
	7.36	7.42	—0.06	7.29	7.35	—0.06	7.28	7.34	—0.06
	6.86	6.92	—0.06	6.80	6.86	—0.06	6.81	6.87	—0.06
	5.77	5.82	—0.06	5.76	5.84	—0.08	5.76	5.77	—0.01
	4.84	4.91	—0.07	4.84	4.93	—0.09	4.84	4.85	—0.01
	3.84	3.91	—0.07	3.85	3.90	—0.05	3.84	3.85	—0.01

1) ITANO, A., Berichte d. Ohara Institute etc. IV, I, 19, 1929.

Table IX. (continued.)

Buffer solution.	H_2			Q.			Qh.		
	N / 10	Saturated.	Difference of PH.	N / 10	Saturated.	Difference of PH.	N / 10	Saturated.	Difference of PH.
CLARK'S	8.76	8.83	—0.07	8.69	8.74	—0.05	8.13	8.14	—0.01
	8.37	8.42	—0.05	8.29	8.33	—0.04	7.85	7.91	—0.06
	7.88	7.93	—0.05	7.88	7.92	—0.04	7.50	7.55	—0.05
	7.14	7.17	—0.03	7.11	7.16	—0.05	7.02	7.05	—0.03
	5.96	6.03	—0.07	5.95	6.00	—0.05	5.98	6.04	—0.06
	5.07	5.14	—0.07	5.09	5.15	—0.06	5.12	5.16	—0.04
	4.03	4.10	—0.07	4.05	4.10	—0.05	4.03	4.06	—0.03
Average			—0.064			—0.058			—0.037
Mean error			±0.0016			±0.0018			±0.0025

As the above table indicates, slightly larger PH values were obtained by the simplified, saturated calomel electrode in all the buffer solutions, viz. PH 0.06 in chain with the H_2-electrode and quinhydrone, and PH 0.04 with hydro-quinhydrone electrode respectively. The simplified, saturated calomel electrode was six months old after it was prepared.

Summary and Conclusions:

1. The comparative tests of quinhydrone and hydro-quinhydrone electrodes were carried out under various conditions, namely in presence of glucose, alcohol, ammonia and other salts.

2. Under all the conditions tested, the quinhydrone electrode seems to be satisfactory providing that the readings are taken soon after the quinhydrone is added viz. within one minute.

3. On the average, the lower PH values are obtained by the hydro-quinhydrone especially in PH > 7. But in the solutions PH < 7, very similar results were obtained by all the electrodes.

4. The quinhydrone electrode is prefered to the hydro-quinhydrone since the manipulation is simpler.

5. The simplified, saturated calomel electrode gives slightly larger PH values in all cases viz. PH 0.06 in chain with the H_2- and quinhydrone electrodes and PH 0.04 with the hydro-quinhydrone electrode respectively. The differences are so small that the simplified, saturated calomel electrode may be used for the most of the biological investigations.