tassium salts when the data were plotted to the axes log k (specific conductance) *versus* $1/T$. Only one slope was observed for thallous halides except in the case of thallous iodide, which undergoes a change of crystal form. In the light of recent high-temperature transference experiments, it is concluded that in the polar lattices only the positive ion conducts at lower temperatures, but that both ions conduct at higher temperatures. Change from uni-ionic to bi-ionic conduction seems to be characteristic of, and limited to, high-melting polar salts. Heats of liberation of the conducting ions in the various lattices have been calculated from the corresponding slopes. Within the sodium and potassium series heats of liberation are proportional to the melting points.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

A STUDY OF THE CALCIUM AMALGAM ELECTRODE IN DILUTE AQUEOUS SOLUTIONS[1]

BY RUSSEL JOHN FOSBINDER

RECEIVED SEPTEMBER 10, 1928 PUBLISHED MAY 6, 1929

The determination of the calcium-ion activities in dilute solutions of calcium salts by the measurement of the electromotive force of a cell of the proper type is interesting for two reasons. First, the electrochemical behavior of calcium amalgams against aqueous solutions of calcium salts apparently discloses many peculiarities not exhibited by other amalgam electrodes. Second, calcium-ion concentration is a matter of considerable importance in physiological chemistry.

Byers[2] attempted to determine equilibrium conditions in a system of calcium hydroxide and sodium carbonate by means of the electromotive-force method. Neuhausen and Marshall[3] studied the electrolyte equilibrium in the blood by the use of amalgam electrodes. Lucasse[4] measured the potentials of cells with and without liquid junctions to determine transference numbers and activity coefficients of the alkaline earth chlorides. Drucker and Luft[5] made a careful and critical study of the calcium electrode in solutions of calcium. Kirk and Schmidt[6] attempted to measure the calcium-ion activities in solutions of certain protein salts but stated that their technique was entirely inadequate.

[1] This communication is based on a thesis submitted for the degree of Doctor of Philosophy at the University of Wisconsin in 1928 under the direction of Farrington Daniels.

[2] Byers, THIS JOURNAL, **30**, 1584 (1908).

[3] Neuhausen and Marshall, *J. Biol. Chem.*, **53**, 365 (1922).

[4] Lucasse, THIS JOURNAL, **47**, 743 (1925).

[5] Drucker and Luft, *Z. physik. Chem.*, **121**, 307 (1926).

[6] Kirk and Schmidt, *J. Biol. Chem.*, **76**, 115 (1928).

The purpose of this investigation was to develop an apparatus and a technique suitable for the accurate determination of the effective calcium-ion concentration in dilute solutions, and also to study the possibility of applying the electrode in determining the state of calcium in solutions of physiological importance.

The Determination of Activity Coefficients of Calcium Chloride.— The activity coefficients of calcium chloride were determined from 0.01–3.3 M in order to become familiar with the technique involved in the operation of the calcium electrode. The apparatus used and the method of procedure for the cells were, in general, similar to those described by Lucasse.[4] The calcium amalgam was prepared electrolytically according to the method prescribed by Neuhausen.[7] In making the determinations one cell was always filled with 0.0099 M calcium chloride, while the concentration of the solution in the other cell was varied at will. It was found that it was not necessary to have the solutions flowing, provided the readings were taken immediately after the amalgam flow was started. By such a procedure steady, reproducible potentials were obtained.

In Table I are given the results, where m is the number of moles of salt dissolved in 1000 g. of solvent, E is the electromotive force generated in the cell and γ is the activity coefficient.

TABLE I

DATA OBTAINED FOR CELLS OF THE TYPE Ag | AgCl | $CaCl_2$ (m) | Ca_xHg | $CaCl_2$ (m = 0.0099) | AgCl | Ag

m	E	γ	m	E	γ
0.0099	0.00000	(0.716)	0.3069	0.11735	0.481
.0435	.04870	.572	.7158	.15605	.577
.0628	.06070	.550	1.2081	.18289	.689
.0781	.06885	.534	1.5378	.20232	.871
.0897	.06685	.494	1.9833	.21702	.974
.1411	.08940	.499	3.2702	.27665	2.820

For a concentration cell without liquid junction containing a bi-univalent salt, the electromotive force, activity coefficient and concentration are related according to the equation

$$E = \frac{RT}{NF} \ln \left(\frac{\gamma_1 \, 2^{2/3} m_1}{\gamma_2 \, 2^{2/3} m_2} \right)^3 = \frac{3RT}{2F} \ln \frac{\gamma_1 \, m_1}{\gamma_2 \, m_2}$$

In calculating the values of the activity coefficient, it was assumed, as Lucasse assumed, that γ is 0.716 at 25° for the 0.0099 M $CaCl_2$ solution. Introducing the numerical values, the equation finally takes the form

$$\log \gamma_1 = 11.27E + (7.84695 - 10) - \log m_1$$

Taking the activity coefficients at round molal concentrations and comparing the values obtained with those of Lucasse, it is seen that they are in excellent agreement up to 1 M.

[7] Neuhausen, THIS JOURNAL, 44, 1945 (1922).

TABLE II

ACTIVITY COEFFICIENTS

m	(Obs.)	(Lucasse)	m	(Obs.)	(Lucasse)
0.01	(0.716)	(0.716)	0.20	0.481	0.480
.02	.655	.655	.50	.502	.499
.05	.567	.569	.70	.560	.560
.07	.539	.541	1.00	.685	.709
.10	.517	.516	2.00	1.150	1.521

The Determination of Activity Coefficients of Calcium Ions.—The problem of determining individual *ionic* activities by electromotive-force measurements involves the determination of liquid junction potentials, and although these potentials can be reduced to small values, they are not known with certainty. While individual ionic activities are of less value from the thermodynamic point of view than the activities of the ionized salts, they are of great importance in many biological and chemical fields where specific ion effects are considered.

There also exists the problem of determining a reference activity, since in working with amalgam electrodes it is difficult to extend the dilution sufficiently in order to take a_1 to be very small. However, in the concentration range studied (0.001–0.01 M) one can calculate the value of a reference activity by employing the equation developed by Debye and Hückel. The limiting expression for the activity coefficient of an ion in aqueous solution at 25° is $-\log_{10}\gamma = 0.505z^2\sqrt{\mu}$, where γ is the activity coefficient, z the valence of the ion, and μ the ionic strength. Experiments have shown that this equation applies at least approximately up to an ionic strength of 0.01. The ion activity, a, may be obtained from the relation $a = \gamma c$, where c is the stoichiometrical concentration. In this investigation these equations have been employed in calculating the value of the reference activity.

Drucker and Luft[5] attempted to determine the ionic activity of calcium in solutions of calcium sulfate but they stated that the limited solubility of the salt made accurate measurements impossible. As yet no one has attempted to work with amalgam electrodes in dilute solutions, possibly because of the fact that such measurements have been considered to be unreliable. The work of Allmand and Polack[8] on the sodium electrode has been criticized by Michaelis and Kawai[9] who claim that in dilute solutions the electrode functions as a mixed hydrogen and sodium electrode. Allmand and Polack made but very few determinations with dilute solutions, the most dilute being 0.01 molal.

In the course of developing an electrode suitable for use with dilute solutions, preliminary studies indicated the following facts. (1) A single electrode is preferable because double dropping electrodes are very difficult

[8] Allmand and Polack, *J. Chem. Soc.*, **115**, 1020 (1919).

[9] Michaelis and Kawai, *Biochem. Z.*, **163**, 1 (1925).

to adjust properly. (2) There is an optimum amalgam concentration for a definite range of solution concentration. (3) The effect of electrode surface area is to be considered. (4) The solutions must be air free. (5) Small particles must be kept from depositing on the electrode surface, as they cause the rapid decomposition of the amalgam.

Experimental Procedure

A high grade of calcium sulfate was repeatedly shaken with conductivity water and filtered. The resulting paste was then used in making up the saturated solution.

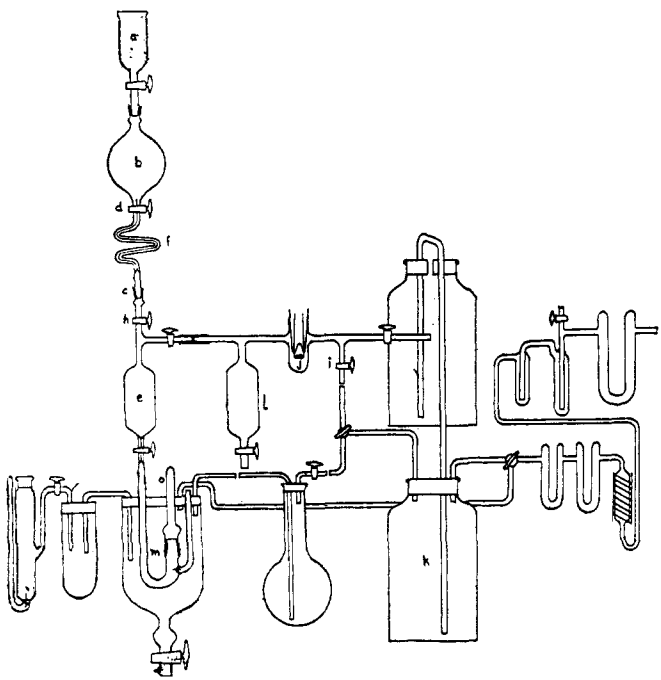


Fig. 1.—Apparatus for calcium electrode.

Other calcium salts used were listed as chemically pure and free from heavy metals. The solutions were made up accurately by weight on the molal basis.

In this part of the investigation the thermostat was discarded in order to facilitate manipulation of the apparatus. The temperature of the room was fairly constant, varying from 23.5–25°, and the errors from temperature fluctuations were less than other errors of measurement.

Commercial hydrogen from a tank was purified by passing it through solid potassium hydroxide, neutral permanganate solution, platinized asbestos at 300°, calcium chloride and phosphorus pentoxide.

The saturated calomel electrode was prepared using pure mercury, mercurous chloride and recrystallized potassium chloride. The bridge was made of saturated potassium chloride in agar-agar.

The apparatus employed is shown in Fig. 1. The entire system was first evacuated, then hydrogen from k was allowed to enter the system. Next a weighed quantity of metallic calcium was introduced into b and the apparatus again evacuated, and a weighed amount of mercury was allowed to flow into b from a. That part of the system which was above the ground-glass joint c was removed and the calcium was shaken with mercury to effect amalgamation. The apparatus was then reassembled and the system below the stopcock d was repeatedly evacuated and flushed out with hydrogen and finally evacuated. The stopcock d was opened and the amalgam allowed to flow from b into the reservoir e through the capillary filter f, which removed any oxide impurities. The stopcock h was then closed and pure hydrogen from k allowed to enter the system through i, which was then closed. The compartment j contained phosphorus pentoxide and a platinum glower to remove residual oxygen and water. The leveling bulb l was used to keep a pressure of hydrogen over the amalgam. The electrode m was fitted with a close-fitting cap o which was removed after the solution was introduced into the cell. With this type of apparatus the amalgam concentration and the electrode surface area were studied. For a solution concentration range of 0.02–0.001 *M* an amalgam concentration of 0.005–0.015% by weight was found to be satisfactory.

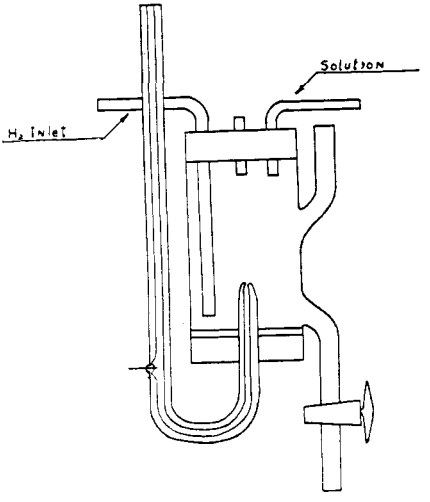


Fig. 2.—Final form of dropping electrode for calcium amalgam.

The first electrode studied was 15 mm. in diameter. In an attempt to measure the potential of an amalgam against a saturated solution of calcium sulfate, large voltage fluctuations were observed due to the decomposition of the amalgam at the surface. The second electrode was 9 mm. in diameter and was equipped with a rotating stirrer which not only agitated the solution but constantly scraped the surface of the amalgam. It was hoped that with this apparatus the solid particles of calcium hydroxide would be removed and the decomposition retarded, but the results were still erratic. Considerably better results were obtained with an electrode 4 mm. in diameter.

The final electrode is shown in Fig. 2. It was of the capillary type with a 1-mm. opening and was used in the experiments recorded in the tables. The stopper in the bottom of the cell was thickly coated with paraffin and inserted at such an angle as to insure the complete removal of the amalgam as it dropped off the tip of the capillary. Before a series of determinations was begun the tip of the capillary was filled with paraffin oil. This procedure was quite necessary as in the absence of the oil the electrode tip very soon became clogged on account of the accumulation of solid calcium hydroxide, necessitating the cleaning of the apparatus. The hydrogen inlet tube served to keep an atmosphere of hydrogen above the solution in the cell and also to agitate the solution during the operation of the electrode.

An inspection of the following tables shows that fairly accurate values of the activity coefficients may be obtained if the electrode surface area is small so that catalytic decomposition of the amalgam by particles of calcium hydroxide is minimized.

For a concentration cell with liquid junction the electromotive force and activity are related according to the following equation

$$E = E_1 - E_2 = \frac{RT}{NF} \ln \frac{a_1}{a_2}$$

or, substituting numerical values

$$E_1 - E_2 = 0.0295(\log \gamma_1 c_1 - \log \gamma_2 c_2)$$

The value of γ_1 is obtained from the Debye–Hückel equation. To illustrate the method of obtaining $-\log \gamma_2$, a calculation is carried out below using data from Table V.

c_1	= 0.0015	c_2	= 0.0031
E_1	= 2.2335	E_2	= 2.2267
$\log \gamma_1$	= −0.158	$\log \gamma_1 c_1$	= −2.980

Substituting these values in the above equation, $-\log \gamma_2 = 0.240$.

Results

All of the results have been calculated for the hypothetical cells $Ca_xHg \mid c_1Ca^{++} \mid$ Satd. KCl in agar-agar $\mid c_2Ca^{++} \mid Ca_xHg$.

The amalgam contained 0.012% of calcium in all cases except in Tables V and VIII, where it contained 0.015%.

TABLE III

ACTIVITY COEFFICIENTS OF CALCIUM IONS CALCULATED FROM E.M.F. OF A CALCIUM AMALGAM ELECTRODE, TAKING FOR REFERENCE THE ACTIVITY IN A DILUTE SOLUTION AS CALCULATED FROM THE DEBYE-HÜCKEL EQUATION

CALCIUM SULFATE. $t = 23.2°$

M	E	ΔE	$-\log \gamma$ (calcd.)	$-\log \gamma$ (obs.)
0.0153	2.1853	0.0107	0.501	0.481
.0153	2.1853	.0107	.501	.481
.0077	2.1907	.0053	.343	.363
.0077	2.1905	.0055	.343	.353
.0038	2.1960	.0000	.250	Ref.
.0038	2.1957	.0003	.250	Ref.
.0038	2.1960	.0000	.250	Ref.

TABLE IV

CALCIUM SULFATE. $t = 23.8°$

M	E	ΔE	$-\log \gamma$ (calcd.)	$-\log \gamma$ (obs.)
0.0153	2.2119	0.0203	0.501	0.479
.0153	2.2121	.0201	.501	.480
.0092	2.2174	.0148	.388	.442
.0092	2.2173	.0149	.388	.439
.0015	2.2320	.0002	.158	Ref.
.0015	2.2322	.0000	.158	Ref.

TABLE V

CALCIUM SULFATE. $t = 24.2°$

M	E	ΔE	$-\text{Log } \gamma$ (calcd.)	$-\text{Log } \gamma$ (obs.)	
0.0153	2.2135	0.0203	0.501	0.479	0.481
.0122	2.2162	.0176	.446	.469	.483
.0092	2.2172	.0166	.388	.382	.391
.0061	2.2196	.0142	.315	.286	.296
.0031	2.2267	.0070	.224	.244	.240
.0015	2.2335	.0000	.158	Ref.	Ref.

TABLE VI

CALCIUM LACTATE. $t = 25.0°$

M	E	ΔE	$-\text{Log } \gamma$ (calcd.)	$-\text{Log } \gamma$ (obs.)	
0.010	2.2105	0.0086	0.349	0.325	0.332
.008	2.2128	.0063	.312	.313	.313
.006	2.2157	.0034	.271	.284	.278
.004	2.2191	.0000	.221	Ref.	Ref.
.002	2.2254	− .0063	.155	.139	.124

TABLE VII

CALCIUM HYDROXIDE. $t = 24.0°$

M	E	ΔE	$-\text{Log } \gamma$ (calcd.)	$-\text{Log } \gamma$ (obs.)	
0.0219	2.2053	0.0155	0.517	0.405	0.377
.0153	2.2113	.0095	.432	.413	.416
.0110	2.2135	.0073	.366	.366	.368
.0044	2.2208	.0000	.232	Ref.	Ref.
.0022	2.2266	− .0058	.165	.119	.....

TABLE VIII

CALCIUM CHLORIDE. $t = 25.0°$

M	E	ΔE	$-\text{Log } \gamma$ (calcd.)	$-\text{Log } \gamma$ (obs.)	
0.0604	2.1912	0.0288	...	0.464	0.464
.0483	2.1927	.0273	...	.414	.....
.0362	2.1985	.0215	...	.483	.481
.0242	2.2023	.0177	...	.442	.439
.0120	2.2091	.0109	0.385	.367	.366
.0085	2.2130	.0070	.322	.350	.355
.0060	2.2159	.0041	.271	.292	.286
.0036	2.2200	.0000	.216	Ref.	Ref.
.0012	2.2303	− .0103	.121	.087	.098

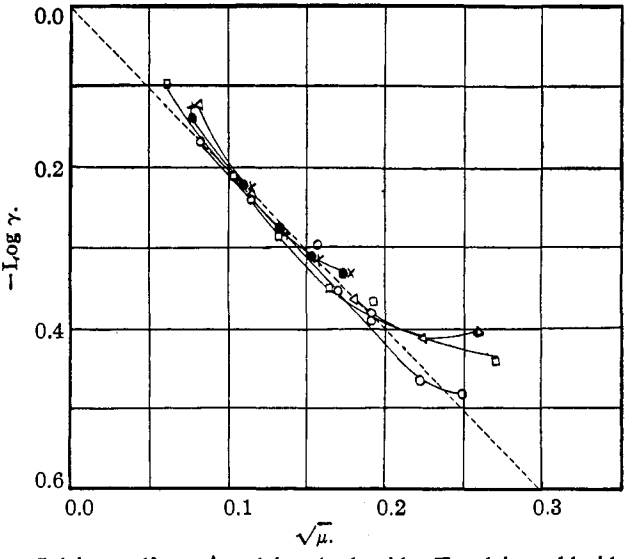
TABLE IX

CALCIUM ACETATE. $t = 23.0°$

M	E	ΔE	$-\text{Log } \gamma$ (calcd.)	$-\text{Log } \gamma$ (obs.)	
0.010	2.2105	0.0086	0.349	0.328	0.333
.008	2.2130	.0061	.312	.313	.313
.006	2.2157	.0034	.271	.284	.287
.004	2.2191	.0000	.221	Ref.	Ref.
.002	2.2252	− .0061	.155	.124	.120

In the preceding tables the value of the reference voltage was taken as the average of several determinations, and the results of duplicate experiments are given in the last two columns. In most cases the reference electrode was taken as the next to the most dilute solution rather than the most dilute solution because the experimental errors in the latter are larger.

The Debye–Hückel equation gives a straight line if $-\log \gamma$ is plotted as ordinates and the $\sqrt{\mu}$ as abscissas, as shown by the dotted line of Fig. 3. Fig. 3 also contains the experimental data for the five different calcium compounds.



○, Calcium sulfate; △, calcium hydroxide; □, calcium chloride; ●, calcium lactate; ×, calcium acetate.

Fig. 3.—Graph showing the relation between the activity coefficient of calcium ions and the ionic strength.

While the equation is a limiting expression and valid only up to the region of an ionic strength of 0.01 (or $\sqrt{\mu} = 0.1$) it may be employed as a first approximation in the region of the concentration studied. The curves obtained for the calcium salts deviate from the theoretical line, the deviations being larger at the higher ionic strength due to the failure of the simple theoretical equation in this region. No attempt is made here to test the validity of the Debye–Hückel expression but it is employed merely as a first approximation to which the experimental data may be compared. The exact curves are probably not significant because the experimental errors are large, but it is seen that they fall fairly close to the Debye–Hückel line. The deviations in the case of the most dilute solutions may probably be attributed to experimental difficulties in this region.

The Effect of Cations on the Potential of the Calcium Electrode.—Electromotive-force determinations of aqueous solutions of calcium salts against calcium amalgam have shown that the electrode is reliable in a concentration of calcium ions comparable to that contained in most physiological systems. The possible application of the calcium electrode to biological problems is at once suggested providing that the potential of the electrode is not seriously disturbed by other electrolytes or proteins present in such solutions.

Neuhausen[10] studied the effect of gelatin and various electrolytes on the reliability of the sodium electrode. From the results of a series of experiments he concluded that the addition of other electrolytes, in general, did not affect the potential of the electrode. To test this point in regard to the calcium electrode a small amount of another electrolyte was added to a solution of calcium sulfate and the effect on the potential was observed. The results of such a series of experiments are contained in Table X.

TABLE X

THE INFLUENCE OF ADDED ELECTROLYTES ON THE POTENTIAL OF A CALCIUM AMALGAM ELECTRODE

Amalgam = 0.012%. t = 23.8°

$CaSO_4$, M	Electrolyte added, M = 0.002	E	ΔE
0.0074	None	2.2186	0.0000
.0074	K_2SO_4	2.1936	.0250
.0074	Na_2SO_4	2.1758	.0428
.0074	$(NH_4)_2SO_4$	2.1583	.0603
.0074	$CuSO_4$	2.0089	.2097
.0074	$FeSO_4$	1.6123	.6063
.0074	Oxalic acid	1.9774	.2412
.0074	Tartaric acid	2.1313	.0873
.0074	$ZnSO_4$	2.1878	.0308
.0074	None	2.2185	.0000

In every case the potential of the electrode was lowered, probably on account of the formation of a mixed amalgam electrode. In the case of iron and copper ions it is likely that the amalgam decomposed rather rapidly. The potassium and sodium ions entered the amalgam and replaced calcium to a certain extent. This replacement is opposed to the normal positions in the electromotive-force series, but the difference between the potential of the sodium or potassium electrode and the calcium electrode is so small that a high concentration of sodium or potassium ions can replace a small number of calcium atoms.[11]

In order to determine if a similar effect can be observed in the case of a physiological solution containing other cations in addition to calcium

[10] Neuhausen, THIS JOURNAL, **44**, 1445 (1922).

[11] Compare G. McP. Smith, *ibid.*, **27**, 540 (1905), and later communications.

ions, a series of samples of fresh dog's blood was prepared. In the following table are contained the results of the determination of the electromotive force of a calcium amalgam against the blood samples. The author is indebted to Professor W. J. Meek of this University for help in these experiments.

TABLE XI

POTENTIALS OF A CALCIUM AMALGAM ELECTRODE IN BLOOD

$t = 25°$

Sample	Treatment	E
0.0153 M $CaSO_4$	.....	1.9543
Blood	Hirudin added	1.7834
Blood	Oxalated	1.8095
Blood	Whipped	1.8112
Plasma	Centrifuged	1.7660
Blood	Whipped	1.8134
0.0153 M $CaSO_4$	.....	1.9537

While the absolute values of the electromotive force are not exact, the data show conclusively the impossibility of applying the calcium electrode in this way to the determination of the calcium-ion concentration in blood or any solution containing cations other than calcium. The lower potentials with blood correspond to an impossibly high concentration of calcium.

It had been hoped that a relation would be found between the effect of various materials on the coagulation of blood and on the calcium-ion activity, but no conclusion can be drawn on account of the complication of other ions.

The Effect of Proteins on the Potential of the Calcium Electrode.—Neuhausen[10] reported that the addition of isoelectric gelatin to solutions containing sodium ions produced no observable effect on the reliability of the sodium electrode. For this reason it was thought that the calcium electrode might be applied in studying calcium proteinate systems.

One hundred grams of 8% isoelectric gelatin was covered with 600 cc. of air-free, saturated calcium hydroxide solution. The system was then immersed in ice water and allowed to stand for one hundred hours. The liquid outside the gelatin was poured off and the gelatin washed with ice water.

Approximately ten g. of Merck's soluble crystallized egg albumin was added to 400 cc. of an air-free saturated calcium hydroxide solution. The mixture was placed in a shaking machine and the shaking continued for a period of seven hours. During this time a slow stream of pure hydrogen was allowed to bubble through the solution. Solutions of calcium caseinate were prepared in a similar manner. The results of electromotive-force determinations of calcium amalgam against solutions of calcium proteinate are given in the following tables.

TABLE XII

INFLUENCE OF GELATIN

Solution	E	$a_{Ca^{++}}$
$Ca(OH)_2$, 0.0074 M	2.2215	0.0046
$Ca(OH)_2$, 0.0074 M	2.2214	.0046
$Ca(OH)_2$ filtrate	2.1870	.0676
$Ca(OH)_2$ filtrate	2.1872	.0676
Ca gelatinate solution	2.1557	.0746
Ca gelatinate solution	2.1560	.0746

TABLE XIII

INFLUENCE OF PROTEINS

$X = 0.015\%$. $t = 24°$

Solution	Concn. of calcium	E	ΔE
Ca albuminate	0.0219	1.9986	0.2210
Ca albuminate	.0044	2.0861	.1335
$Ca(OH)_2$	.0219	2.2196	.0000
$CaCl_2$ + albumin	.0604	1.8416	.3514
$CaCl_2$ + albumin	.0121	1.9532	.2398
$CaCl_2$	.0604	2.1930	.0000
1 cc. $CaCl_2$ + Albumin + 49 cc. $Ca(OH)_2$	.030	2.2055	.0058
49 cc. $Ca(OH)_2$ + 1 cc. $CaCl_2$	.030	2.1113	.0000
Ca caseinate	.0219	1.8100	.4106
1 cc. Ca caseinate + 49 cc. $Ca(OH)_2$	.0223	2.2120	.0086
$Ca(OH)_2$	.0219	2.2206	.0000

The data clearly show that in a solution containing 0.02% or even a smaller amount of protein the reliability of the electrode is destroyed, for the values of "a" and ΔE correspond to an impossibly high concentration of calcium ions.

The alteration in the potential must be due to a change which takes place at the surface of the electrode. To account for this effect the following explanation is advanced. Because of a tendency of the protein to form a film on a metallic surface a coating is formed on the surface of the electrode. This film prevents the ordinary electrode equilibrium from taking place and results in the building up of a large ionic concentration inside the film. Experiments conducted with other metallic electrodes in protein solutions indicated the plausibility of this hypothesis. The results also suggest the reason for the failure of other investigators to develop a technique applicable to the study of calcium solutions containing protein.

The author desires to acknowledge the help given by Professor Farrington Daniels, under whose direction this investigation has been carried out.

Summary

1. Values of the activity coefficients of calcium chloride are given from 0.01–3.3 molal. The values up to a concentration of 1.0 molal agree very well with those obtained by Lucasse.

2. A technique and an apparatus suitable for the determination of the activity coefficients of calcium ions have been developed.

3. Values of the activity coefficients of calcium ions in aqueous solution for five calcium salts have been determined from 0.01–0.001 molal.

4. The investigation has revealed apparently insurmountable difficulties in using the calcium electrode for the determination of calcium-ion activities in physiological solutions. The potential is lowered by the presence of cations, either above or below calcium in the electromotive-force series. The potential is lowered also by the presence of protein.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, LABORATORY OF MICRO-ANALYSIS, WASHINGTON SQUARE COLLEGE, NEW YORK UNIVERSITY]

MICRO-POTENTIOMETRIC DETERMINATION OF REDUCING CARBOHYDRATES

BY JOSEPH B. NIEDERL AND RALPH H. MÜLLER

RECEIVED SEPTEMBER 24, 1928 PUBLISHED MAY 6, 1929

Introduction

Of the various types of methods in existence for the micro-determination of reducing sugars, the copper reduction method with its various modifications is used most extensively.[1]

It has been shown by Daggett, Campbell and Whitman[2] that reducing sugars can be determined potentiometrically. The purpose of this communication is to show that micro-analytical determination can be made by this method and that the method may be simplified by the use of a more convenient electrode than the calomel electrode employed by these authors. The more important carbohydrates have been studied and their reduction values ascertained. One method (semi-micro) permits the determination of 3 to 5 mg. with an accuracy of about $\pm 1\%$, while the other (micro) permits a quantitative determination of as little as 0.05 mg. of reducing carbohydrates.

[1] (a) I. Bang, *Biochem. Z.*, **87**, 27, 248, 264 (1918); (b) **88**, 92, 344 (1918); (c) H. MacLean, *J. Physiol.*, **50**, 168 (1916); (d) *Biochem. J.*, **13**, 135 (1919); (e) P. A. Shaffer and A. F. Hartmann, *J. Biol. Chem.*, **45**, 349, 365 (1921); (f) A. Kowarsky, *Deut. med. Wochschr.*, **45**, 188 (1919); (g) E. Mislowitzer, *Biochem. Z.*, **67**, 168, 217 (1916); (h) S. Zisa, *Chem. Zentr.*, [I] 2611 (1926); (i) S. Rosenthaler, *Arch. Pharm.*, **263**, 518 (1926); (j) G. Fontes and L. Thivolle, *Compt. rend. soc. biol.*, **84**, 669 (1921); (k) O. Folin and Hsien Wu, *J. Biol. Chem.*, **38**, 106 (1920); (l) **41**, 367 (1920); (m) O. Folin and H. Berglund, *ibid.*, **51**, 209 (1922); (n) V. E. Rothberg and F. A. Evans, *ibid.*, **58**, 435, 443 (1922); (o) S. Morgulis and co-workers, *Chem. Zentr.*, [IV] 635 (1923); (p) S. R. Benedict, *J. Biol. Chem.*, **64**, 207 (1926); (q) **68**, 759 (1926); (r) L. Lorber, *Biochem. Z.*, **158**, 158, 205 (1925); (s) E. Komm, *Z. angew. Chem.*, **38**, 1094 (1926); (t) Goiffon and Nepveux, *Compt. rend. soc. biol.*, **83**, 121 (1920); (u) D. Charnass, "Biol. Arbeitsmethoden," Abt. IV, T. 4, p. 1179.

[2] Daggett, Campbell and Whitman, THIS JOURNAL, **45**, 1043 (1923).