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.

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MICRO-POTENTIOMETRIC DETERMINATION OF REDUCING CARBOHYDRATES

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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).

Apparatus.—The apparatus consisted of two vessels filled with Fehling's solution of the same concentrations. Platinum electrodes were immersed in each vessel, the two vessels being connected by an agar agar–potassium chloride bridge. One vessel was heated and the sugar solution added slowly from a buret. The difference in the potential of the electrodes was followed by means of a Leeds and Northrup Type K potentiometer. The Hildebrand[3] arrangement was also used successfully.

Figure 1 illustrates this arrangement as employed in the micro-determination. The vessels in this case were small pyrex tubes. The bridge consisted of a capillary tube of 1 mm. outside diameter, filled with agar agar–potassium chloride gel.

Experimental Part

Three to 5 mg. of the substance is weighed accurately on a micro-analytical balance down to 0.001 mg. (Kuhlmann balance; Becker's micro-analytical balance might also be used) and dissolved in distilled water, one cc. for each mg. The solution is put in the micro-buret. To each of the two vessels 1 cc. of Fehling's solution containing one mg. of copper per cc. is added. A reading is now taken on the potentiometer. Then the Fehling's solution in container A is heated to boiling and a reading taken again.

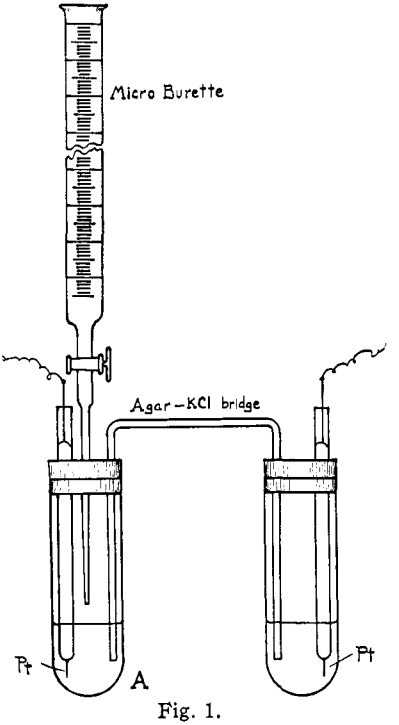

Fig. 1.

A preliminary titration is first carried out as follows: 0.1 cc. of the solution containing the carbohydrate is added from the buret, the mixture boiled and a reading taken again. These additions are continued until 1.2 cc. has been used.

In the region of the end-point the increase in potential is very rapid. The maximum value of millivolts per 0.01 cc. marks the end-point.

The Fehling's solution used was prepared as follows: 3.95 g. of $CuSO_4 \cdot 5H_2O$ in 500 cc. of water, and 19.75 g. of sodium potassium tartrate and 7.40 g. of sodium hydroxide in 500 cc. of water.

The actual determination is carried out in the same way as the previous one, using larger amounts until the end-point is approached. From this point small amounts, not exceeding 0.02 cc., are added and after boiling readings are taken. This is continued over the range ascertained in the first trial.

The change in potential in the vicinity of the end-point was always very large, enabling one to determine it without difficulty. The actual values of the potentials, however, were not as highly reproducible, owing to the fact that the reaction was

[3] Hildebrand, THIS JOURNAL, **35**, 847 (1913).

carried out in an alkaline medium and no particular precautions were taken for constant temperature. A typical titration curve is reproduced in Fig. 2.

For those cases in which less than four mg. is available, the material is weighed out according to Pregl,[4] and placed directly in the titrating vessel. Then Fehling's solution is added as before, boiled and readings are taken on the potentiometer.

The titration itself, however, is completed with standard glucose solution (1 mg. in 1 cc.) in the manner given. The difference in the volume necessary for the end-point gives the amount of carbohydrate present. Naturally a solution (1 cc.) free from other reducing substances might also be used instead of the solids.

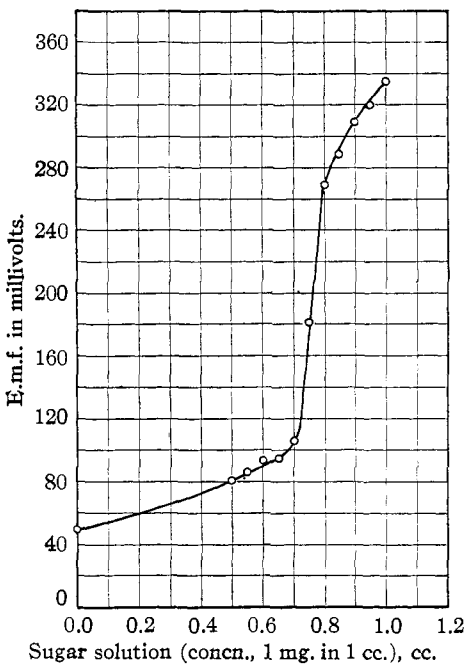

Fig. 2.

Discussion of Results

Under the experimental conditions outlined above, the following empirical standard values were obtained.

1 Mg. of Copper

Sugar	Mg.
d-Glucose	0.635
d-Mannose	.638
d-Galactose	.770
d-Fructose	.679
Maltose	1.019
Lactose	.884
Sucrose (inverted)	.657

1 Mg. of Sugar

	Cu, mg.
d-Glucose	1.575
d-Mannose	1.567
d-Galactose	1.298
d-Fructose	1.472
Maltose	0.981
Lactose	.884
Sucrose (inverted)	1.522

Each of these empirical standard values represents the average of at least six determinations.

Some of the results as obtained by this method by various individuals are given below. The Fehling's solution contained 1 mg. of copper in 1 cc. All the sugar solutions used also contained 1 mg. of reducing carbohydrate in 1 cc. The materials used were "Kahlbaum" chemicals of highest purity.

Since the reducing power of the various carbohydrates changes with the reaction conditions, such as concentration, temperature and oxidizing reagent, the foregoing values do not check absolutely with the figures given in the literature and obtained under different conditions, although

[4] Pregl, "Quantitative Organic Micro-analysis," P. Blakiston's Sons and Co., Philadelphia, 1924.

1 Mg. sample	Cu, mg.	Average	1 Mg. sample	Cu, mg.	Average
d-Glucose	0.630–0.660	0.645	*d*-Fructose	0.675–0.700	0.687
Standard value,	.630– .640	.635	Standard value,	.650– .680	.665
0.635	.630	.630	0.679	.675– .700	.687
	.630	.630	Maltose	1.050–1.100	1.075
	.630	.630	Standard value,	1.000–1.050	1.025
	.620– .640	.630	1.019	1.000–1.025	1.013
	.630– .650	.640		1.000–1.030	1.015
	.625– .650	.638		1.000–1.050	1.025
d-Mannose	0.625–0.650	0.638	Lactose	0.850–0.900	0.875
Standard value,	.625– .650	.638	Standard value,	.850– .900	.875
0.638	.625– .650	.638	0.884	.880– .900	.890
	.600– .700	.650		.880– .900	.890
	.625– .650	.638		.880– .900	.890
d-Galactose	0.750–0.800	0.775	Sucrose (inverted)	0.650–0.675	0.663
Standard value,	.770– .800	.785	Standard value,	.650	.650
0.770	.750– .780	.765	0.657	.650– .675	.663
	.750– .780	.765			
	.750– .775	.760			

they correspond in the order of the reducing power with those obtained by related methods. The comparison is as follows.

Order of red. power	Potent. titr.	E. Wein	Lewis–Benedict	Folin–Wu	Fehling	Knapp	Sachsse
1	Glucose	Glucose	Glucose	Glucose	Glucose	Fructose	Fructose
2	Sucrose	Sucrose			Sucrose	Sucrose	Sucrose
3	Mannose		Mannose	Fructose			
4	Fructose		Fructose	Galactose	Galactose	Glucose	Glucose
5	Galactose		Galactose	Mannose	Fructose	Galactose	Galactose
6	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose	Lactose
7	Maltose	Maltose	Maltose	Maltose	Maltose	Maltose	Maltose

In carrying out numerous titrations by the method set forth herein where the Fehling's solution is just brought to boiling after each addition of the carbohydrate solution, no appreciable differences in the results were noted, whether the solution was boiled up 5, 6, 7 or 8 consecutive times. Perhaps in such diluted solutions the time factor no longer is of marked influence.

Since the various sugars have different standard values, it is evident that in a pure sample also the qualitative nature of the carbohydrate may be ascertained by comparing the titration curve obtained with the standards. For example, if a pure sample weighing 1 mg. consumes 0.981 mg. of copper it can only be maltose, etc.

The authors desire also to thank Mr. Nathan Ambinder for carrying out a large number of titrations.

Summary

Micro-potentiometric methods for the quantitative determination of reducing carbohydrates have been described.

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