cies of plants; and the possibility exists that it may play an important role in nitrogen metabolism.

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The Determination of Chloride by Use of the Silver-Silver Chloride Electrode[1]

Martin Chanin[2]

Laboratory of Thoracic Surgical Research,
City of Hope Medical Center, Duarte, California

Accepted methods for the determination of chloride may be grouped mainly under four headings: (a) gravimetric; (b) titrimetric, with color indicators; (c) potentiometric, with titration; and (d) optical. Attempts have been made to adapt these methods so that they may be used in dilute solutions and with biological materials, but such adaptions are usually time consuming and not too accurate. This paper describes an improved potentiometric method for chloride ion determination. The time required for a complete determination should be no more than one minute.

A consideration of the Nernst equation shows that, with the proper electrode pair, it should be possible to set up a concentration cell for the determination of almost any ion. The voltage will not be affected by the presence of biological material, and there should be minimal interference from other ions. Tremblay (*1*), Neuhausen and Marshall (*2*), Duxbury (*3*), and Katsu (*4*) decided that the method has only very limited application. One of the greatest difficulties has been drifting. Drifts have been eliminated in the present investigation by making all dilutions with a buffer solution of pH 7. These drifts are due to the changing pH, which is caused by the absorption of atmospheric CO_2.

There is a daily change in the voltage developed by known chloride concentrations, so it has not been possible to compute values from theoretical considerations (*1, 2*). It is necessary to plot a reference curve each time the electrodes are used, but because the voltage is a logarithmic function of concentration, only two points are needed. These are plotted on semi-log paper, and the straight line that connects them is the desired curve. Dilutions should be made so that the final unknown chloride concentration is in the range of 0.1 to 1.0 milliequivalents per liter. Also, the concentrations of at least two different dilutions of the same unknown should be determined. There should be insignificant variation when the original concentration is computed.

TABLE 1. Millivolt readings for various concentrations of chloride with the silver-silver chloride electrode.

Concentration (Milliequivalents per 1000 ml)	Reading (mv)
2.0	245
1.5	239
1.0	230
0.9	227
0.8	225
0.7	222
0.6	219
0.5	215
0.4	209
0.3	203
0.2	194
0.1	178

The electrodes used in the present investigation are a mercury-mercurous sulfate reference electrode (which has provision for a saturated potassium sulfate bridge built into it),[3] and a silver-silver chloride electrode.[4] The latter electrode is a rod of solid silver, and is shipped with a silver chloride coating already on it. This coating must be renewed completely before it can be used, as unexplained drifts occur with an insufficiently pure silver chloride coating.

The following method for renewing the silver chloride has worked best. Scour the silver electrode with cleansing powder until no purple brown color can be seen. Rinse with distilled water and then connect to the negative pole of a 1½-v dry battery. Connect a platinum electrode to the positive pole, immerse the electrodes in dilute ammonium hydroxide, and allow current to flow for about 1 min. Remove the electrode, wipe off any discolored material, and rinse with distilled water.

Coating with silver chloride is now effected by electrolyzing in a saturated solution of reagent-grade potassium chloride. Connect the clean silver electrode to the positive pole of a 1½-v dry battery, a platinum electrode to the negative pole, and allow current to flow for 20 sec. Without rinsing, place the freshly prepared silver-silver chloride electrode in distilled

TABLE 2. Millivolt readings and chloride concentrations for different dilutions of blood serum.

Dilution	Concentration (Milliequivalents per 1000 ml)	Reading (mv)
	1.0	233
	0.1	180
1–100	1.14	236
1–1000	0.114	183

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[2] The author acknowledges the consultative assistance of Wendell H. Griffith, Chairman of the Department of Physiological Chemistry at the University of California Medical School at Los Angeles.

[3] Beckman 1170-9.

[4] Beckman 1264.

water and allow it to stand in contact with the excess chloride ions that have been carried over from the saturated solution, for 30 min. Finally, rinse the electrode with distilled water and place it in distilled water until it is used. The efficiency of the electrode is not impaired if the silver-chloride coating is not uniform. It is important, however, that care be taken to insure that it does not dry out. Electrodes that have been prepared in the above manner have been used for several weeks before they have been recoated.

Voltage measurements can be made, most conveniently, on the millivolt range of a pH meter.[5] However, extreme accuracy requires that a slide wire potentiometer be used. To determine chloride concentration, connect the silver-silver chloride electrode to the positive binding post of the measuring instrument, the reference electrode to the negative binding post, and immerse the two electrodes in the unknown solution. The voltage developed across the electrodes is plotted on the concentration curve, and the unknown concentration is read directly. The electrodes should be rinsed with distilled water and blotted dry with a cleansing tissue between each two determinations. Table 1 shows the voltages developed by pure chloride solutions of various concentrations. Table 2 shows the voltages and the equivalent chloride concentrations for two different dilutions of the same blood serum. Several determinations of chloride in thoracic duct fluid and in buffered extracts of dry muscle tissue have been made. In every case, no drift was observed. The chloride concentration in thoracic duct fluid is identical with that of the corresponding blood serum, and the chloride of the muscle checks with values that have been found in the literature. Urine chloride has also been determined.

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[5] The Beckman Model G was used.

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A Simple Method for the Calculation of Electrode Potentials in Polarography[1]

Gabor Markus

Department of Physiology, Stanford University, School of Medicine, Stanford, California

In polarographic work it is frequently necessary to convert applied voltage to electrode potential. This is accomplished by subtracting the product of current *i*, and total circuit resistance, *R*, from the applied voltage. This product is referred to as the *iR* factor. Its evaluation is a cumbersome operation and involves the separate experimental determinations of the current and of the resistance. The proposed method is simpler, because the *iR* factor can be obtained directly from a polarogram employing the low-voltage range customary in polarography;[2] thus the *iR* factor can be evaluated without the separate determination of either *i* or *R*.

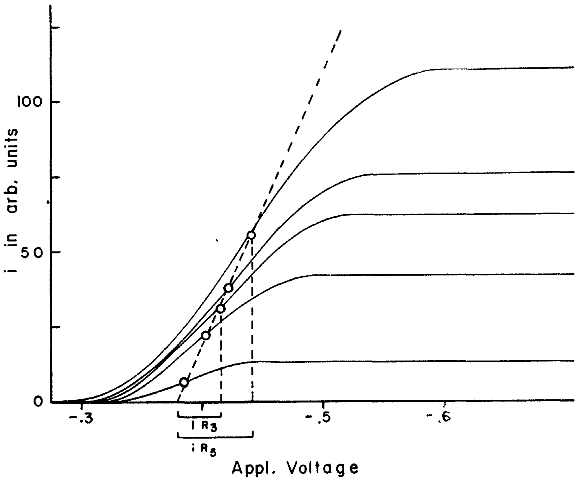

Fig. 1. Five concentrations of $Pb(NO_3)_2$ buffered to pH 6.8.

The electrode potential corresponding to a given ratio of oxidant to reductant is a characteristic constant of a given substance and is independent of the concentration. An easily measured potential of this kind is the half-wave potential, where the ratio of oxidant to reductant is unity for all substances that are reducible or oxidizable at the electrode. If, however, current-voltage curves of the same substance are

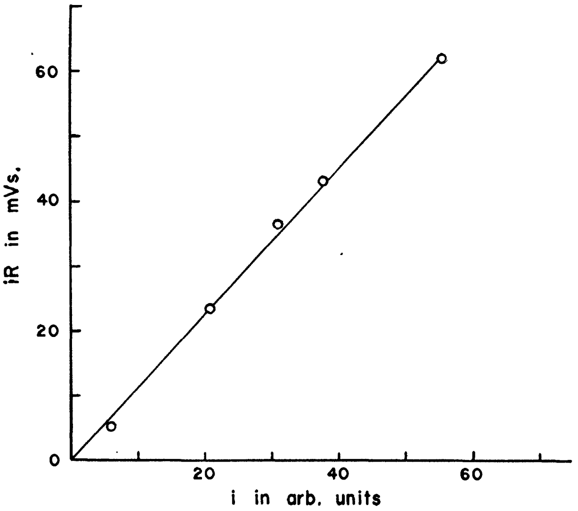

Fig. 2. Calibration curve constructed on the basis of Fig. 1.

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[1] Carried out on a contract between the Office of Naval Research (N6 ONR 25137) and Stanford University.

[2] Müller's determination of the *iR* factor involves the application of higher voltages. O. H. Müller, *The Polarographic Method of Analysis*, p. 97. Easton, Pa.: Chemical Education Pub., 1951.