A POLAROGRAPHIC MICROMETHOD FOR THE DETERMINATION OF BLOOD CHLORIDE*

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Because of the inherent sensitivity of the dropping mercury electrode when used for the quantitative analysis of inorganic ions, the present study was undertaken to determine whether sufficient accuracy and simplicity of operation could be attained to justify its employment as a micromethod for the determination of chloride in blood. Since it was desired to work with such samples as can be conveniently obtained from small laboratory animals for serial analyses, a method has been developed for 0.02 and 0.05 ml. samples of whole blood, plasma, and serum.

Kolthoff and Miller (1) have shown that the concentration of chloride ion in a solution can be determined by measurement of the diffusion current of the anodic depolarization wave produced at the dropping mercury electrode. Using 0.1 M potassium nitrate as supporting electrolyte, they found that the diffusion current was directly proportional to chloride concentrations between $10^{-4}$ and $2 \times 10^{-3}$ equivalent per liter. The electrode reaction is

$$2\text{Hg} + 2\text{Cl}^- \rightarrow \text{Hg}_2\text{Cl}_2 + 2e$$

In applying their findings to the development of a method for the determination of chloride in blood, the general procedure used for the preparation of the sample has been to remove proteins, provide a supporting electrolyte, and dilute the sample about 1:100 in as few operations as possible. Phosphotungstic acid is used for the dual purpose of precipitating proteins and of acting as a supporting electrolyte. The concentration of chloride in the clear supernatant solution obtained on centrifugation is determined by a single measurement of the diffusion current at a fixed applied voltage.

Apparatus

A manual apparatus similar to that described by Kolthoff and Lingane (2) was used in the determinations described in this report. A Leeds and Northrup student model potentiometer was used to set the potential of the

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dropping electrode. The diffusion current was measured with a reflecting galvanometer and scale. The sensitivity of the galvanometer was adjusted to approximately 0.013 μa. per mm., and the instrument was overdamped to reduce the amplitude of the current oscillations. The drop times of the capillaries used were between 2 and 4 seconds.

A compact electrolysis cell-reference electrode assembly was devised which contributed greatly to the practicability of the method for routine use (Fig. 1). The assembly consists of a saturated mercurous sulfate reference electrode (+0.4 volt versus saturated calomel electrode), an electrolysis cell of about 1 ml. capacity, and a dropping mercury electrode, all aligned permanently on a ring-stand. The cell is drained by means of the stop-cock at the bottom; thus it need not be removed for filling or rinsing. A water jacket containing about 100 ml. encloses the electrolysis cell to
minimize the effect of any change in room temperature during a series of analyses. It is not necessary to know the temperature of the cell, since the unknown solutions and standards are compared at the same temperature.

Reagents—

1. Phosphoric acid solution, approximately 0.15 M. Into a 1 liter volumetric flask transfer 10 ml. of phosphoric acid (sp. gr. 1.7) and dilute to the mark with distilled water.

2. Sodium tungstate solution, 3.0 per cent. Dissolve 30 gm. of reagent grade sodium tungstate in distilled water and dilute to 1 liter.

3. Stock standard potassium chloride solution, 0.2 M. Dissolve 14.91 gm. of dried, analytical reagent grade potassium chloride in distilled water and dilute to 1 liter.

4. Diluted standard potassium chloride solutions. Transfer to 100 ml. volumetric flasks 55, 50, 45, 40, and 35 ml. of the stock standard potassium chloride solution. Dilute to 100 ml. to prepare standards containing 110, 100, 90, 80, and 70 m.eq. of chloride per liter, respectively.

5. Working standard solutions. To 100 ml. of a phosphotungstic acid solution, prepared from 80 ml. of the approximately 0.15 M phosphoric acid solution and 20 ml. of the 3 per cent sodium tungstate solution, add 1.0 ml. of one of the diluted standard potassium chloride solutions. Since the dilution of the chloride standards in these working standard solutions is the same as the dilution of the blood samples, each will correspond to a chloride concentration in the blood sample equivalent to the chloride concentration of the standard solution from which it was prepared.

Procedure

The following technique is used in the preparation of 0.05 ml. samples of whole blood, serum, or plasma. The sample is delivered into 4.0 ml. of the approximately 0.15 M phosphoric acid in a test-tube, and 1.0 ml. of the 3 per cent sodium tungstate solution is added to precipitate proteins. The solution is thoroughly mixed and then centrifuged approximately 1 minute at 2000 R.P.M. The clear supernatant solution is then ready to be transferred to the electrolysis cell.

When 0.02 ml. samples are used, the procedure is exactly the same except

1 The whole blood is drawn directly from the animal into the pipette and delivered into the diluting solution, before clotting can occur, to obviate the necessity for an anticoagulant. For serum determinations the blood is drawn into a soft glass capillary tube, and one end of the tube is sealed in a Bunsen flame at some distance from the blood column (to prevent rupture of red cells). After centrifugation the capillary is scratched with a diamond pencil at the junction of the serum and red cells. The tube is broken off and the serum drawn directly into a pipette from the capillary.
that the quantities of 0.15 M phosphoric acid and 3 per cent sodium tungstate used are reduced to 1.6 and 0.4 ml., respectively.

Since it is unnecessary to record complete current-voltage curves for routine analyses, a polarimetric procedure is used; i.e., the current is measured at a single, fixed, applied voltage. A voltage within the diffusion current of the chloride wave is chosen at which increments in chloride concentration produce directly proportional increments in current. For the procedure described here, a setting of -0.06 volt against the saturated mercurous sulfate reference electrode was found suitable (+0.34 volt versus saturated calomel electrode). Oxygen need not be removed from the solutions to be analyzed, since it is reduced at potentials negative to the chloride wave.

The cell is rinsed once with the solution to be analyzed, refilled, and the minimal and maximal galvanometer readings are recorded. A sufficient number of the standard chloride solutions in phosphotungstic acid to cover the range of chloride values encountered in the material used are run simultaneously. The mean deflections for the blood dilutions are referred to the calibration provided by the mean deflections for the chloride standards.

**EXPERIMENTAL**

The accuracy of the method was evaluated by the following studies.

1. Calibration curves show a direct proportionality of diffusion current to chloride ion concentration.

2. The precision of the method as shown by the reproducibility of galvanometer readings with successive samples of the same chloride solution is generally within 1 scale division. At the usual setting of the galvanometer shunt a variation of 1 scale division amounts to 0.33 m.eq. per liter. In several series of ten successive samples of the same blood dilution it was found that the greatest difference in current readings within each series was not more than 2 scale divisions.

3. The agreement of duplicates was essentially the same whether the determinations were made on whole blood, serum, or plasma. A series of thirty-four pairs of 0.05 ml. samples showed an average difference between duplicates of 0.55 per cent, the greatest difference encountered being 1.5 per cent. Thirty-one pairs of 0.02 ml. samples showed an average difference of 0.75 per cent, the greatest difference, with one exception, being 1.5 per cent.

4. In nine experiments in which the equivalent of 16.0 m.eq. per liter of chloride as potassium chloride was added to 0.05 ml. samples of plasma and whole blood the mean error in recovery was 0.9 m.eq. per liter; the maximal error was 1.5 m.eq. per liter.

5. The values obtained by this method applied to 0.05 ml. samples of serum, plasma, and whole blood were compared with the values obtained
by the iodometric titration method of Sendroy, as modified by Van Slyke and Hiller (3), with 1 ml. samples. A series of twenty-eight parallel determinations in duplicate was carried out. The mean deviation between the values obtained by the two methods was 1.0 m.eq. per liter. 86 per cent of the polarographic values agreed with Sendroy values within 2.0 m.eq. per liter, and none differed by as much as 3.0 m.eq. per liter.

**DISCUSSION**

The foregoing study of the accuracy of this method indicates that it is adequate for following changes in blood chloride concentration. The procedure is simple and rapid, since preparation of the blood consists merely in the measurement and dilution of the sample, followed by centrifugation to remove the protein precipitate. The use of the polarimetric technique (a single current reading at a fixed applied voltage) and of the electrolysis cell assembly illustrated here contributes further to the simplicity of the method in routine use. Thus this method differs from the established volumetric micromethods in that a single galvanometer reading is substituted for the usual microtitration of an aliquot with a standardized solution.

By the use of a very small electrolysis cell this method could be adapted to the determination of much smaller quantities of chloride than those used here, with no other modification of the technique. To illustrate this a small electrolysis cell (Fig. 2) was devised which permitted the determination of...
chloride concentration in 0.05 ml. of the phosphotungstic acid dilution of chloride. Calibration curves obtained with 0.05 ml. portions of 5, 7, 9, and $11 \times 10^{-4}$ M chloride in phosphotungstic acid solution showed a direct proportionality between diffusion current and chloride concentration. The quantity of chloride ion in these samples was 0.89, 1.24, 1.60, and 1.95 $\gamma$, respectively. With these small samples it was noted that the galvanometer reading remained constant at its maximal deflection for only about four current oscillations, and then drifted to lower values. The diffusion current readings for successive samples of the same solution generally agreed within 2 scale divisions (about 0.01 $\gamma$ of chloride).

While the above investigations were in progress, the work of Schönholzer (4) on the polarographic determination of chlorides in biological materials came to the attention of the authors. His technique is similar to that presented in this paper in that the chloride concentration in the blood dilution is determined by measurement of the diffusion current. However, complete current-voltage curves are recorded for each sample, and in this procedure the proteins are not removed from the solution to be analyzed. The sample is merely diluted in 0.1 N sulfuric acid, which acts as the supporting electrolyte. The accuracy of the procedure is stated to be ±2 per cent. Because of the simplicity of this method of preparing the sample, its reproducibility and accuracy were studied with the apparatus and technique described in this paper. The reproducibility of galvanometer readings with successive samples of the same dilution of whole blood or plasma was the same as that found for phosphotungstic acid dilutions. A series of determinations in duplicate was carried out on nine whole bloods and nine serums with 0.05 ml. samples of both the sulfuric and phosphotungstic acid dilutions, together with parallel determinations on the same samples by the Sendroy macromethod (1 ml. samples). The mean deviation from the Sendroy values when sulfuric acid was used as the diluting fluid was 1.5 m.eq. per liter; with the phosphotungstic acid the mean deviation was 0.9 m.eq. per liter. The slightly better agreement of the latter procedure with the macromethod thus was not remarkable, even though on a theoretical basis it would seem better practice to make the blood dilutions as nearly like the chloride solutions used for calibration as possible by the removal of proteins.

There are a number of substances, not normally present in blood or present only in inappreciable quantities, which also depolarize the dropping mercury electrode at the voltage used in the determination of chloride. The only such substances likely to be encountered in experimental work are thiosulfate, thiocyanate, bromide, and iodide. When any of these is present, its diffusion current will be added to that of the chloride at $-0.06$ volt versus the saturated mercurous sulfate electrode.

The presence of such interfering substances can be detected readily by
comparing the current of the blood dilution at a potential just negative to the beginning of the chloride wave (−0.2 volt versus the saturated mercurous sulfate electrode, or +0.2 volt versus the saturated calomel electrode) with that of one of the standard chloride solutions or of a blank solution of phosphotungstic acid. In the absence of interfering ions the current will be identical for all three solutions at this voltage; the presence of an interfering ion in the diluted blood will give rise to a current greater than that of a standard chloride solution or a blank. However, such interfering substances are not likely to be encountered in blood unless they have been purposely administered, and for practical routine work the procedure is much more rapid when only a single current measurement is made.

SUMMARY

A method has been developed for the determination of chloride in 0.02 and 0.05 ml. samples of blood by means of polarimetric analysis with the dropping mercury electrode. It is rapid, simple, and accurate.

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