Potentiometric Determination of Chloride in Biological Fluids

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Potentiometric titration of chloride with silver nitrate has been a standard procedure for many years, and the theoretical considerations involved have been clearly set forth by Kolthoff & Furman (1931). However, methods based on this principle have been little used in biological work, in spite of their attractive possibilities. Lehmann (1939) seems to have been the first to describe a method suitable for clinical work; more recently, Norberg (1949) has

When silver nitrate is added to a solution containing chloride ions, the concentration of silver ions in the solution is at first very low. As more silver nitrate is added, the silver-ion concentration rises slowly, and then more rapidly as the equivalence point is reached. When this point is passed, and all the chloride is precipitated as silver chloride, silver ions accumulate rapidly.

These changes can be followed potentiometrically, by immersing a silver electrode and an indifferent electrode in the solution and measuring the potential difference between them. If this p.d. is plotted against the volume of silver nitrate added, a sigmoid curve, like that illustrated in Fig. 1, *A*, is obtained. It has been shown (Kolthoff & Furman, 1931) that the equivalence point either coincides with the centre of the steepest portion of the curve, or is separated from it by a negligible distance.

EXPERIMENTAL

Apparatus

The apparatus consists of a microburette (0.25 ml. or 2 ml.), a stirring device, indifferent and silver electrodes, and an instrument to indicate the potential difference. The latter must present a high resistance to the electrodes, since the passage of a current through the cell causes electrolysis, which may vitiate the results unless the amount is kept very small. In the arrangement described by Lehmann (1939), the instrument used was a slide-wire potentiometer of the usual null-point type, requiring the adjustment of a dial for every reading. In the present arrangement this has been replaced by an electronic millivoltmeter which has a moderately high input resistance (5 MΩ) and at the same time provides continuous indication of the input voltage on a robust moving-coil type of instrument. This greatly simplifies the procedure of titration and gives visual indication of the approach of the end point. This instrument is described in the Appendix. If a direct reading electronic millivoltmeter is already available, e.g. in the form of a pH meter, it will serve the purpose admirably; the pH meter type of instrument, however, has a much higher input impedance than is necessary for the present purpose and is likely to be more costly in consequence.

The silver electrode, as in Lehmann's method, is simply a piece of 20 s.w.g. silver wire dipping into the solution, but for the indifferent electrode another modification has been introduced. Lehmann used a calomel or quinhydrone electrode, connected to the titration vessel by a potassium nitrate agar bridge. Such bridges are inconvenient, being somewhat troublesome to prepare and maintain; when not in use, the free end must be kept immersed in saturated

Fig. 1. Titration curves of 0.2 ml. 0.1 N-NaCl (*A*); 0.2 ml. normal human plasma (*B*); and 1 ml. normal human urine (*C*). Abscissae (ml. of 0.1 N-AgNO₃) for (*A*) and (*B*) at lower border of figure and for (*C*) at upper border.

proposed a similar procedure, differing from Lehmann's mainly in the arrangements for measuring potential difference. Neither of these methods exploits to the full the great convenience and speed which is possible with potentiometric titrations; the method here described is an adaptation of Lehmann's, but is considerably quicker and more convenient. Chloride can be determined in 0.2 ml. of plasma or urine, and when plasma is being analysed removal of protein is unnecessary.

potassium nitrate solution, as, if exposed to the air, the agar shrinks, leaving a small 'dead space' at the end of the tube which readily traps a bubble of air and thus breaks the continuity of the circuit. By an ingenious device due to Willard & Boldyreff (1929) the use of bridges may be avoided and one of the elements dipping into the titration vessel eliminated. A piece of platinum wire is sealed into the wall of the burette below the tap, and the outer end of this connected by a wire to the voltmeter. The platinum is thus immersed in a solution of constant composition, and takes up a constant potential with respect to it. The circuit is completed by dipping the burette tip (which should be of fine bore, to minimize diffusion) under the surface of the titration mixture.

Some means of stirring the mixture must be provided. The author uses the stream of air from a vibrating-reed pump of the type used to aerate the water of aquaria. A mechanical shaking device, or a magnetic stirrer could no doubt be used instead.

The type of burette used depends on the size of sample being analysed. If plasma, or small amounts of urine, are being analysed, 0.2 ml. is taken and a Conway burette (capacity 0.25 ml.) has proved very convenient. If larger amounts of urine are available a sample of 1 ml. can be taken and this will give a sharper end point; a larger burette is then required and the author uses a 2 ml. burette graduated in divisions of 0.02 ml. In both cases the burette requires the minor modification of a platinum wire sealed in below the tap.

Reagents

Silver nitrate (0.1 N); acetic acid 50% (v/v); octan-2-ol ('capryl alcohol').

Procedure

In the classical method of potentiometric titration, a graph is prepared of every titration and the end point determined by inspection. In this method, however, the conditions are so constant that this is unnecessary; the end point occurs at the same potential with every titration, whether plasma, urine or standard NaCl solution is being titrated. With the author's apparatus, this potential lies between 190 and 200 mV. AgNO_3 is added until this potential is shown by the meter which is used exactly like an indicator in an ordinary titration. Protein does not alter the end-point potential: in its presence, the curve is not quite so steep, but the centre of the steep portion remains at the same potential (Fig. 1, B).

Although the end-point potential with the author's instrument has proved quite constant, this potential should be determined independently for other instruments by constructing titration curves in the usual way.

For plasma, the procedure is as follows: acetic acid (5 ml. of 50% v/v) is pipetted into a flat-bottomed glass tube (2×7.5 cm.). Plasma (0.2 ml.) is added from a washout pipette, together with 1 or 2 drops of octan-2-ol (to prevent foaming). The meter is adjusted for sensitivity and zero, and the glass tube is placed in position under the burette (Fig. 2). A potential of the order of 350 mV. should now be registered on the meter. AgNO_3 (0.1 N) is next added from the burette: at first there is little change in potential and the solution can be run in rapidly, but as the end point is approached the potential begins to change more quickly and when it has reached 300 mV. the AgNO_3 should be added more cautiously.

With the Conway burette volumes of 0.001 ml. can be added accurately and as Fig. 1 shows, there is no difficulty in deciding on the end point with additions of this order. A short time is required for the potential to change after each addition of silver, and at or near the end point it is necessary to wait for some 10–15 sec. before reading the meter, otherwise the end point will be overshoot. For this reason, the 'magic eye' type of indicator is unlikely to be satisfactory, as it gives insufficient warning of the approach of the end point. If x ml. of 0.1 N- AgNO_3 nitrate are used in the titration, then the sample contains $100x/0.2 = 500x$ m-equiv. of chloride per l.

Fig. 2. Arrangement of apparatus for titration.
(Scale one-half actual size.)

The procedure for urine is very similar. If only small amounts are available, a sample of 0.2 ml. can be analysed as described above with entirely satisfactory results. However, if larger amounts are available, 1 ml. samples give a sharper end point (Fig. 1, C) and are more convenient to pipette. Acetic acid (5 ml.) is used as before and the mixture is titrated with 0.1 N- AgNO_3 from a 2 ml. burette, until the meter reads 190–200 mV. If x ml. of 0.1 N- AgNO_3 are required for titration, then the sample contains $100x$ m-equiv. of chloride per l.

RESULTS

Accuracy

The accuracy of the method was tested in two separate ways: by recovery experiments after the addition of known amounts of chloride to plasma

and urine, and by parallel determinations of chloride in plasma and urine by the present method and by Van Slyke & Hiller's (1947) modification of Sendroy's (1937) iodometric method.

Recovery experiments

Plasma. A number of samples of human plasma were pooled and dialysed in a cellophan sac against distilled water for 24 hr., the water being changed after the first 12 hr. At the end of this time the plasma contained less than 0.5 m-equiv./l. of chloride. Known amounts of sodium chloride were then added to samples of this dialysed plasma, and the samples titrated by the usual procedure. The results are shown in Table 1.

Table 1. *Recovery of chloride added to dialysed plasma and to urine low in chloride*

(Results of chloride recovered expressed to nearest 0.5 m-equiv./l.)

Chloride added (m-equiv./l.)	Chloride recovered (m-equiv./l.)	Error (%)
Dialysed plasma		
80	80	0
100	99.5	-0.5
120	119	-0.8
Urine		
50	50	0
100	101.5	+1.5
150	150.5	+0.3
200	201	+0.5
250	250	0

Urine. A similar procedure was used; urine low in chloride was obtained from a case of cardiac failure, and known amounts of sodium chloride were added. The results are shown in Table 1.

Parallel determinations

The results of chloride estimations of four samples of plasma and five samples of urine by the present method and by the method of Van Slyke & Hiller (1947) are shown in Table 2. The plasma samples were obtained from a normal subject in the course of an infusion of 10% (w/v) sodium chloride solution, which accounts for the high chloride figures.

In the course of preliminary experiments with Van Slyke & Hiller's method, it was found that the results obtained with plasma, when tungstic acid was used to precipitate proteins, were consistently 1–2 m-equiv./l. higher than those obtained by the electrometric method. When, however, picric acid was used instead of tungstic acid, good agreement between the two methods was found. In the plasma determinations by the method of Van Slyke & Hiller quoted in Table 2, picric acid was used.

Table 2. *Parallel determinations of chloride in plasma and urine by electrometric titration and by the method of Van Slyke & Hiller (1947)*

Chloride content (m-equiv./l.)		
	Electrometric method	Van Slyke & Hiller method
Plasma 1	104.5, 104.5	104.8, 105
2	124.6, 125.2	125, 125
3	123.6, 122.6	122.8, 123.2
4	113.8, 113.8	113.7, 114
Urine 1	220.2, 222.2	220.2, 220.5
2	273, 273	271, 271.8
3	13.8, 13.8	12.6
4	137, 137.5	137.3, 137.5
5	118.7, 118.7	117.8, 118.1

Van Slyke & Hiller noted this difference, but were unable to account for it; they showed that contamination of the sodium tungstate with chloride was not the cause. They also found that a reference plasma chloride method (Van Slyke, 1923–4) gave results agreeing better with the picric acid procedure than with the tungstate method. The results reported here suggest that when the method of Van Slyke & Hiller is being used for plasma, and when results of the highest accuracy are required, picric acid should be used for the removal of proteins in preference to tungstic acid.

Interfering substances

The presence of phosphate or sulphate in concentrations up to 100 mm has no effect on the results. Oxalate can be used as anti-coagulant without introducing any error apart from that due to red-cell shrinkage.

Bromide and iodide both interfere, giving results which are too high by the amount of extra halide present. Thiosulphate also interferes since the solubility of silver thiosulphate upsets the relation between silver-ion concentration and amount of chloride present.

SUMMARY

A rapid and accurate method of determining chloride in small amounts (0.2 ml.) of biological fluids is described. The method depends upon the electrometric titration of the chloride in the sample with silver nitrate, using a directly indicating electronic millivoltmeter.

My attention was drawn to the possibility of an electrometric method for chloride in plasma by Dr J. N. Hunt. Some of the earlier development of the method was done in collaboration with Dr A. H. James. I am grateful to Mr P. W. Roberts for making me familiar with the virtues of the cathode-follower as an impedance transformer, and to Messrs Ionic Instruments Ltd. for certain improvements on the original circuit.

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APPENDIX

Circuit of millivoltmeter

The circuit of the electronic millivoltmeter is shown in Fig. 3. The input voltage is applied across the grids of a double triode valve connected as a double cathode-follower. The output voltage appears across the cathodes and is measured by a moving coil meter in series with an adjustable

an input of 500 mV. to the valve, and transfers the meter to its normal working position across the cathodes. R_9 is now adjusted until the meter again reads 500 mV. S_1 is finally turned to its 'uppermost' position and the instrument is ready for use. R_2 and R_9 should require only occasional adjustment.

Fig. 3. Circuit of amplifier (for references see list of components).

resistance. The input impedance is 5 M Ω , but because of the low output impedance of a cathode-follower circuit, appreciable current (50 μ A.) can be drawn by the meter without interfering with the input circuit. After an initial 'warming-up' period of 10 min. or so, the instrument exhibits very great inherent freedom from 'drift', and the zero and sensitivity remain unchanged when the mains voltage is varied between 180 and 250. R_{11} is the zero adjustment, and R_9 the sensitivity control. Calibration is carried out as follows. S_1 is operated so that the contacts represented in Fig. 3 as being lowermost are made. (The meter is now connected directly, via R_8 , to the voltage dividing chain across B .) R_2 is adjusted until the meter reads 500 mV. S_1 is now turned to its middle position; this applies

Component values are as follows: R_1 , 60 Ω ; R_2 , 20 Ω ; R_3 , 30 Ω ; R_4 , 5 M Ω ; R_5 , 10 000 Ω minus meter resistance; R_6 , R_7 , 500 000 Ω ; R_8 , 4000 Ω ; R_9 , 2500 Ω ; R_{10} , 20 000 Ω ; R_{11} , 500 Ω ; R_{12} , R_{13} , 200 Ω ; R_{14} , 75 000 Ω ; R_{15} , 2.5 Ω ; C_1 , 16 μ F. electrolytic, 350 V. working; S_1 , 4-pole, 3-way wafer switch, Yaxley type; S_2 , on-off toggle switch; B , 1.5 V. dry battery, size U2; M , 0-50 microammeter, 3.5 in. scale, marked 0-500 mV. (Pullin).

All resistors and potentiometers should be wirewound, except R_4 , R_8 and R_7 , which should be of the 'cracked-carbon' type.

An instrument constructed to the above specification can be obtained from Ionic Instruments Ltd., 14D Princes Mews, London, W. 2.