ELECTROMETRIC TITRATION OF CHLORIDE IN SMALL VOLUMES

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The potentiometric end-point in the titration of chloride with silver nitrate depends upon the fact that the concentration of Cl^- ions determines the potential of a silver wire coated with silver chloride which dips into the titration vessel. This chloride electrode is connected through a voltmeter with a reference electrode which completes the circuit, and when the end-point is reached there is a very rapid change of potential between the electrodes, of the order of 100 mV or more. This method is not only convenient but also promises to be practicable on the micro-scale where there is insufficient thickness of solution for the colour change of an indicator to be perceptible.

Advances in the field of potentiometric titration have been frequently reviewed (see, for example, Furman, 1954). The possibilities of the potentiometric end-point in microanalysis were first exploited by Schwarz & Schlösser (1933), who were able to measure 1 μg. of chloride with an accuracy of 'a few per cent'. Linderström-Lang, Palmer & Holter (1935) reduced the scale of operation to 0·4 μg. with an error of $\pm 4{\cdot}5\%$. Cunningham, Kirk & Brooks (1941) measured 0·5 μg to $\pm 2\%$. Since the present work was first taken in hand, Shaw (1955) has published a method accurate to $\pm 1\%$ in less than 1 μg.

While the principle of the method is the same in all cases the form of apparatus used has been very different, particularly in respect of the titration vessel and the reference electrode. Schwarz & Schlösser used a small flask and an $Ag/AgNO_3$ non-polarizable electrode. Cunningham *et al.* titrated in an open dish using a silver-mercury amalgam wire as reference electrode. Linderström-Lang *et al.* used a silver wire sealed into the tip of the burette, and a similar arrangement was also adopted by Sanderson (1952) and by Shaw, the latter carrying out the titration in a drop resting upon a hydrofuge surface.

In the course of this work we have developed two methods. The first is on the 1 μg. scale and has much the same accuracy as Shaw's method, with a possible slight advantage in the form of the burette used. The second method operates on the scale of 10^{-3} μg. (or, the scale of 10^{-3} μl.) and introduces a new feature in that the Ag^+ ions are added not as a solution from a burette but by electrolysis of the silver electrode, the quantity of electricity passing being the measure of the chloride present.

THE FIRST METHOD

The apparatus required for the first method is illustrated in Fig. 1. A Scholander-type burette is used, containing 0·01 N-$AgNO_3$, one unit on the micrometer head corresponding to 0·078 μl. A platinum wire is sealed into the delivery limb. The titration vessel is a small Pyrex tube 0·5 cm. in internal diameter and 2 cm. long, carried upon a platform which can be raised so that the delivery limb of the burette dips into and supports the titration vessel. Also dipping into the titration vessel is the silver wire electrode and a silica capillary from which compressed air is bubbled to stir the contents of the vessel. The voltmeter is similar in all but minor detail to that described by Sanderson but has a higher input impedance of 10 MΩ.

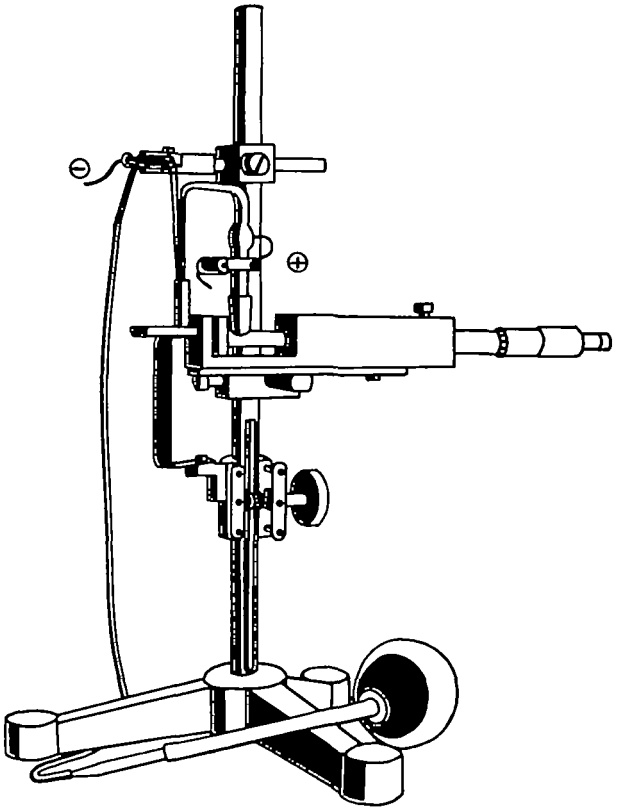

Fig. 1.

About 20 μl. of 50 % acetic acid are first measured into the titration vessel. The sample is taken up in a micropipette and washed out with the acetic acid in the vessel. The vessel is then placed in position and the burette is read. The voltmeter is adjusted so that the needle is near the top of the scale. As the end-point is approached the silver nitrate is added in steps of one unit. After each step the contents of the titration vessel are thoroughly mixed and the potential is recorded. This is continued until the end-point has been passed. Voltmeter reading can be

plotted against burette reading and the end-point is given by the point of inflexion of the curve. At the end of the titration the platform is lowered, the titration vessel is removed and the dipping assembly is washed with a jet of distilled water. The burette is so mounted that it can be rotated about the axis of the stand and is thus separated from the rest of the assembly for purposes of refilling. A single determination can be completed in 5 min.

The method has been tested with pure NaCl solutions and with biological fluids ranging from 0·2 to 10 μl. in volume and from 10 to 500 m.equiv./l in chloride concentration; the standard deviation of a series of similar titrations is less than ± 1 %. Sanderson has shown that protein does not interfere with the potentiometric end-point and that no error is introduced by the presence of phosphate, sulphate or oxalate. To this list it has been possible to add citrate and benzene-sulphonate.

THE SECOND METHOD

The possibility of reducing the scale of the potentiometric titration is limited by two factors: (*a*) the deterioration in the sharpness of the end-point as the dilution is increased, and (*b*) the increase in volumetric error as the volumes of sample and titrant are reduced. Schwarz & Schlösser pointed out that the sharpness of the end-point depends upon the solubility product of silver chloride and showed that the sharpness could be improved in the presence of high concentrations of alcohol or acetone; in this way they were able to work with solutions as dilute as 10^{-4} N. In biological work, however, the dilution factor need not be serious, since the body fluids of animals have chloride concentrations in the range 10–500 m.equiv./l., for which an adequately sharp end-point can be expected. It is more usually the small quantity of fluid available which calls for reduction in scale. A technique has already been developed (Ramsay, 1950) whereby small volumes of fluid are kept under liquid paraffin and from which samples can be drawn up to a mark in a fine silica pipette. Although the volume of the pipette—of the order of 10^{-3} μl.—is only known approximately, the volumes delivered are reproducible to ± 3 % or better. Given some means of measuring the length of the column in the silica tube, e.g. a travelling microscope, it is clear that this technique forms a basis for an attempt to reduce the scale of the method by about one thousand-fold. In some early attempts the sample was deposited from one pipette upon the end of a fine silver wire, under liquid paraffin, and contact was made between it and a reference electrode through a fine capillary. Another pipette was filled with silver nitrate and used as a burette. Titrations carried out in this way were reproducible within $\pm 2{\cdot}5$ % (standard deviation). But while this method was being developed it occurred to us that as an alternative to adding silver nitrate from a burette it would be possible to liberate Ag^+ ions by passing a current through the system and that this current could be used to charge a condenser whose voltage would then be a measure of the amount of Ag^+ ion liberated.

Consider the following system:

$$\mathrm{Ag} \mid 0{\cdot}1\,\text{N-}\mathrm{AgNO_3} \vdots 0{\cdot}1\,\text{N-}\mathrm{NaNO_3} \vdots \underset{\text{(small volume)}}{0{\cdot}1\,\text{N-NaCl}} \mid \mathrm{AgCl/Ag}.$$

Since silver can act as a reversible electrode, not only to Ag^+ ions but also (when coated with silver chloride) to Cl^- ions, the system forms a concentration cell which (liquid junction potentials disregarded) is capable of developing a potential of about 450 mV.

When the two silver electrodes are connected through an external circuit current will flow and Ag^+ ions will be liberated from the Ag/AgCl electrode until their concentration around it reaches 0·1 N. The first Ag^+ ions to be liberated will combine with the Cl^- ions of the 0·1 N-NaCl, and when all the Cl^- ions have been precipitated the potential will have fallen to about 225 mV. If a condenser is included in the external circuit the titration current will develop a voltage across it. For convenience we will refer all calculations to a 'standard sample', 10^{-3} µl. of 0·1 N-NaCl. The quantity of electricity passed during titration of the standard sample is $9{\cdot}65 \times 10^{-6}$ coulomb. The voltage across the condenser cannot be greater than the voltage given by the concentration cell; at the end-point this is 225 mV., from which it can be calculated that the capacity of the condenser must be at least 43 µF.

In practice this arrangement would have the disadvantage that with the effective titration potential falling to zero the approach to the end-point would be intolerably slow. This can be overcome either (*a*) by increasing the capacity of the condenser or (*b*) by including an additional source of potential in the circuit. The capacity may be increased either directly by adding more condensers, which would inconveniently increase the size of the apparatus, or indirectly by electronic devices which would need very carefully stabilized supplies. The stepping-up of potential is not without its dangers, since if the potential across the cell exceeds 1·7 V. there may be electrolysis of water as well as of silver. We have compromised by including a 1·5 V. dry battery in the circuit, which has made it possible to reduce the condenser bank to 20 µF.

Another important matter is the current taken by the voltmeter and the leakage currents of the whole system, considered in relation to the quantity of electricity involved and the time taken to complete the titration. The quantity of electricity is $9{\cdot}65 \times 10^{-6}$ coulomb, and a reasonable estimate of the titration time is 2 min. For the error to be within 1 % the leakage must not exceed $9{\cdot}65 \times 10^{-8}$ coulomb in 2 min., which corresponds to a current of 8×10^{-10} amp. The grid current of electrometer valves is of the order of 10^{-12} amp. or less, but the leakage current of the condenser bank approaches the critical value. The condensers used are ordinary radio components, 2 and 4 µF., 800 V, d.c. working, specially selected for high leakage resistance. Measurements made on these condensers indicate a leakage current of $0{\cdot}81 \times 10^{-10}$ amp./µF./V. If the capacity is 20 µF., the voltage developed in titration of the standard sample will be 487 mV., and under these conditions the leakage current will be $7{\cdot}9 \times 10^{-10}$ amp. Special condensers with plastic insulation, having a leakage resistance about one hundred times higher than paper condensers, are prohibitively expensive.

The details of the apparatus are shown in Fig. 2. A layer of silicone D.C. 1107 is deposited upon the silver wire and a clean surface is produced by cutting off the

end. The clean surface is wettable, whereas the silicone-covered surface is non-wettable; the droplet is therefore stable when in contact with the end of the wire. Contact is made between the sample and the reference electrode through a fine glass capillary. From its opening, about 20 μ in diameter, the capillary rapidly widens to about 1 mm. internal diameter. This part of the system is filled with N-$NaNO_3$ set in agar; it continues into a vertical tube containing N-$NaNO_3$ (without agar) under a layer of liquid paraffin. A similar vertical tube contains a silver wire in N-$AgNO_3$ under liquid paraffin, and contact is made between the two tubes with a N-$NaNO_3$-agar bridge. The reason for this complication is that the penetration of Ag^+ ions into an agar gel results in the darkening of the gel; with the arrangement just described this penetration is limited to the bridge which is discarded and replaced when necessary. The resistance of the reference electrode system is of the order of 7 MΩ.

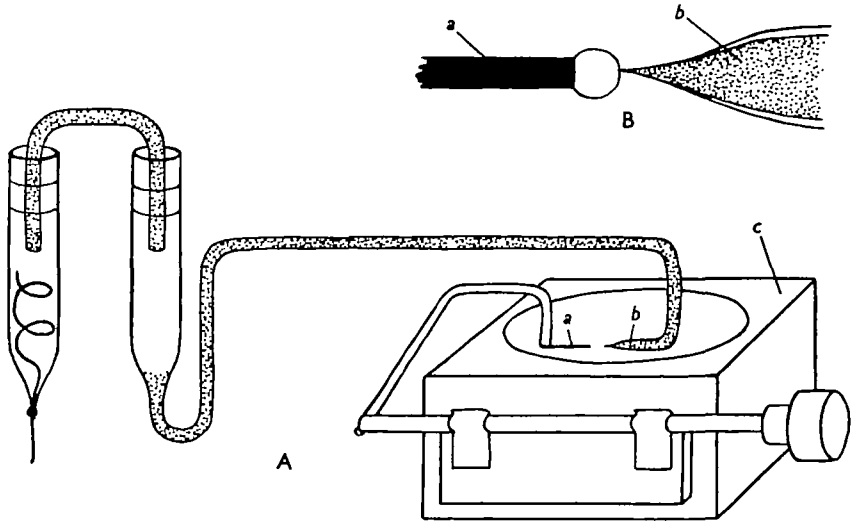

Fig. 2. A, general arrangement. B, silver wire, sample and reference electrode enlarged. *a*, silver wire electrode; *b*, capillary of reference electrode; *c*, Perspex bath.

The liquid paraffin in which the sample is submerged is contained in a Perspex bath mounted upon a small table about 5 in. above the bench. This makes it possible to place a binocular microscope in position to view the sample with transmitted light. The brass arm carrying the silver wire is friction-filled to the side of the bath and provides for up and down movement (by rotation) and for movement from left to right (by sliding). Adjustment in the fore and aft direction is provided for on the mounting of the reference electrode; but once this has been correctly positioned it can be left while the silver electrode is removed for cleaning and replaced after each titration.

N-H_2SO_4 is used instead of acetic acid since the latter is soluble in liquid paraffin. This acid is first drawn up to the mark in the silica pipette, a small length of liquid paraffin is drawn in and then the sample of chloride-containing solution is drawn

up to the mark. The tip of the pipette is brought up to the silver wire and its contents are driven out. It is convenient to bring up the pipette upon the right-hand side, and for this reason the silver wire is directed to the right. The reference electrode must be mounted upon the left so as to clear the pipette. These requirements account for the somewhat twisted aspect of the arrangement shown in Fig. 2*b*.

The electrical circuit is shown diagrammatically in Fig. 3. Switches provide for nominal capacities of 4, 8, 12, 16 and 20 μF. and for series resistances of 1, 6, 11 and 21 MΩ. When the button is pressed the titration current is switched on, and when it is released the cell is connected to the voltmeter. The condenser-bank shorting switch is operated by lifting and turning the press-button. In view of the low voltages involved all contacts are made of silver and have a slight wiping action.

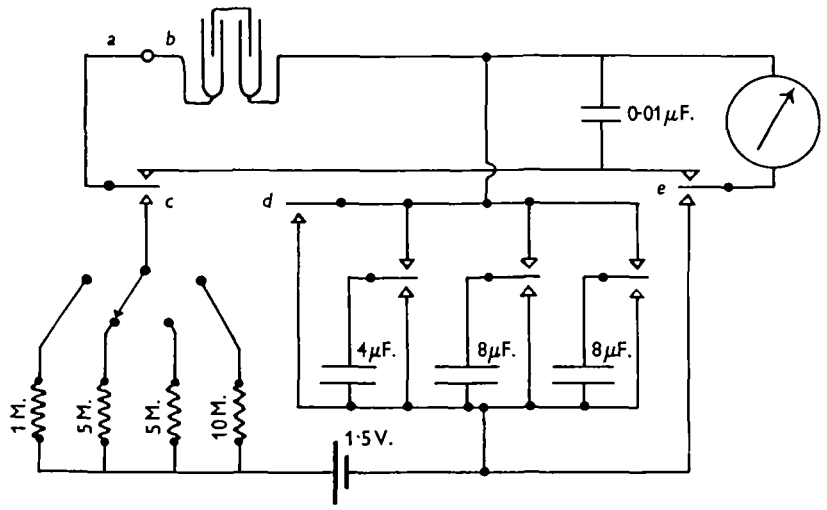

Fig. 3. Circuit diagram. *a*, silver wire electrode; *b*, reference electrode; *c*, titration button; *d*, condenser shorting switch; *e*, voltmeter switch.

They are mounted upon Perspex to ensure a high leakage resistance. The 0·01 μF. condenser across the leads from the cell to the voltmeter serves to stabilize the instrument while it is open-circuited during the passage of the titration current.

For the voltmeter an instrument with a galvanometer scale reading directly in millivolts is to be preferred to one in which the galvanometer is used for null-point only. We have made use of a Pye Universal pH Meter, taking a current of 10^{-12} amp.

Since the Ag^+ ion cannot be added in small steps of equal size (owing to variations in electrical resistance and effective potential), the end-point cannot be conveniently ascertained by the graphical method. Instead, it is necessary to titrate to a definite potential which can be arbitrarily chosen provided that it lies in the steep part of the curve; in the present work a potential of 240 mV. has been taken

as the end-point. The potential registered at the beginning of the titration is a useful indicator of the concentration of chloride present, e.g.

100 m.equiv./l. approx. 450 mV.,
10 m.equiv./l. approx. 390 mV.,
1 m.equiv./l. approx. 330 MV.

The series resistance can be adjusted to the operator's taste as the titration proceeds. The titration button is pressed for a few seconds at a time, and after it is released the galvanometer pointer falls abruptly, rising to a steady value in 2–3 sec. When the end-point is reached the voltmeter is switched to the condenser bank and the voltage is recorded. The silver wire is then removed, washed with a jet of distilled water and replaced. When the apparatus is not in use the silver wire is replaced with a platinum wire loop containing a drop of N-$NaNO_3$, into which the end of the capillary is inserted.

Table 1

Volume of sample (μl. $\times 10^{-3}$)	Concentration (m.equiv./l.)	Quantity (μg. $\times 10^{-3}$)	Capacity (nominal) (μF.)	Voltmeter readings (mV.)	Mean (mV.)	Standard deviation (%)
1·64	20	1·16	4	777, 780, 776, 775, 773	776	±0·33
1·64	5	0·29	4	201, 199, 201, 201, 202	201	±0·55
1·64	1	0·08	1	161, 160, 170, 166, 157	163	±3·3
0·545	100	1·93	8	688, 704, 686, 701, 693, 688, 694, 694, 696, 691	693·5	±0·84
0·145	100	0·51	4	344, 356, 364, 370, 355	358	±2·85

A representative set of data is presented in Table 1. From this table it can be seen that the standard deviation is $< \pm 1$ %, provided that the volume of the sample is not less than $0{\cdot}545 \times 10^{-3}$ μl. and that its concentration is not less than 5 m.equiv./l.; that is to say, the method will measure 10^{-4} μg. of chloride with a standard deviation of $< \pm 1$ %.

The relation between amount of chloride and condenser charge is found to be linear, as expected from theory.

The volume of the silica pipette has hitherto been determined from measurements of its length and diameter made with an eyepiece micrometer, no allowance being made for refraction or for departure from circular cross-section. The measurement of diameter is not believed to be better than ± 10 %, which implies a volume error of ± 20 %. The method was therefore first envisaged as a comparative one, in which the titre of an unknown sample would be compared with the titre of a sample of known solution taken with the same pipette. The success of the method and the general agreement with theory embolden us to believe that the charge on the condenser is in fact an absolute measure of the amount of chloride titrated. If this is true, we have an effective means of determining the volume of the pipette, since the electrical measurements can be carried out with some precision. The volumes quoted in Table 1 have been arrived at in this way.

As already mentioned it has been shown that there is no error due to the presence of protein when the titration is carried out on the macro-scale, and there is no

reason to believe that protein will affect the electrolytric titration just described. This was checked nevertheless by comparison of human serum with 100 mM/l. NaCl, using both first and second methods, and agreement was found to within the expected limits of error. The presence of protein does affect the second method, however, in the following way. After some half dozen titrations of protein-containing samples it is observed that the resistance of the system has increased, and that when the titration button is released the galvanometer pointer does not swing up quickly and smoothly to its new value, but rises irregularly over a period of several seconds. The symptom is such as would be produced by a barrier to diffusion between the surface of the electrode and the body of the sample, and if the cake of silver chloride is dislodged the course of titration returns to normal at once. The same symptom appears in the absence of protein if the cake of silver chloride in the electrode is allowed to dry in air. In all these cases, of course, the remedy is to produce a clean surface of silver by a fresh cut.

SUMMARY

1. Two methods of titrating chloride with Ag^+ ion using the potentiometric end-point are described.

2. The first method is conventional in that silver nitrate is added from a burette. It deals with volumes down to 0·2 μl. and can measure 1 μg. of chloride with an error of $< \pm 1\%$ (standard deviation).

3. According to the second method Ag^+ ion is added by passing a current through a silver electrode in series with a condenser. The charge developed on the condenser is a measure of the amount of chloride titrated. This method deals with volumes down to $0{\cdot}5 \times 10^{-3}$ μl. and can measure 10^{-4} μg. of chloride with an error of $< \pm 1\%$ (standard deviation).

4. As far as is known these methods are not susceptible to interference from other substances likely to be present in biological fluids.

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