# CCCXXXIV.—*The Determination of Chlorides with the Highest Accuracy.*

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The aim of the investigations which form the subject of the present article was the development of a method for the determination of chlorides in samples containing as little as 5 mg. of chloride, with the further requirements that the limit of error should be smaller than 0·1% of the total chloride and that the analysis should be rapid.

Space does not permit a review of the numerous papers on the quantitative estimation of chlorides; with the exception of gravimetric methods, the literature list at the end of this article covers most of the field. Only one method, that of Sørensen (1902), is more than sufficiently precise, but this requires large samples and is very time-consuming.

*Presentation of the Method.*—The present method is essentially a small-volume Volhard titration in which the excess of silver nitrate is reduced to the order of 1—2% and all operations are carried out in a single vessel. Both the sample for analysis and the silver nitrate solution are measured out with an accuracy of about 1 part in 15,000 by means of the adjustable syringe-pipettes described in the preceding paper. The excess of silver nitrate is titrated with ammonium [*] thiocyanate (0·05 to 0·2*M*, depending on the particular requirements) by means of the Rehberg microburette (*Biochem. J.*, 1925, **19**, 270). Since the adjustment of the amount of silver nitrate to the amount of chloride in the sample for analysis is a prime necessity, the method may be most simply described by the consideration of a specific example.

A solution, approximately 0·160*M* in chloride, is to be analysed by means of, say, approximately 0·150*M*-silver nitrate solution and 0·1*M*-ammonium thiocyanate (standardised to within 1%). For the delivery of the silver nitrate solution a syringe-pipette is available which delivers exactly 1·7000 c.c. (the temperature is not specified because the syringe-pipettes have hardly any temperature coefficient). Accordingly, 0·255 milli-equivalent of silver nitrate will be delivered. The Rehberg microburette, in the usual form,[†] has a total range of 100 c.mm. and, if the end-point is to be reached at about the middle of the microburette, a total of 0·05 × 0·10, *i.e.*, 0·005 milli-equivalent, of thiocyanate will be delivered and a similar amount of silver nitrate will be used up with the formation of the insoluble silver thiocyanate. There should be, then, about 0·250 milli-equivalent of chloride in the sample, and this amount will be contained in 0·250/0·160 or 1·5625 c.c. if the concentration is 0·160*M*. A syringe-pipette is set to deliver about this volume and the exact delivery is determined by weighing the amount of distilled water delivered (see the preceding paper for the details of this simple and rapid adjustment).

The foregoing may be termed the preliminary to analysis and the syringe-pipettes, having been set and calibrated, serve to analyse any solution which is within 2% of 0·160*M* in chloride. The subsequent analytical procedure may now be described in detail. The chloride solution is measured out into the Pyrex centrifuge titration tube shown in Fig. 1 by means of the appropriate syringe-pipette, and the silver nitrate solution is added from the other syringe-pipette. The tube is shaken for an instant and about 1 c.c. of concentrated chlorine-free nitric acid is added in a fine stream (as from a pipette with a narrow-bore tip), washing the sides of the tube completely. The tube is now stoppered with the glass capsule (*stopper* in Fig. 1) as used here in the Rehberg micro-chloride method. After standing over-night, or at least 2 hours, in the dark, the tube

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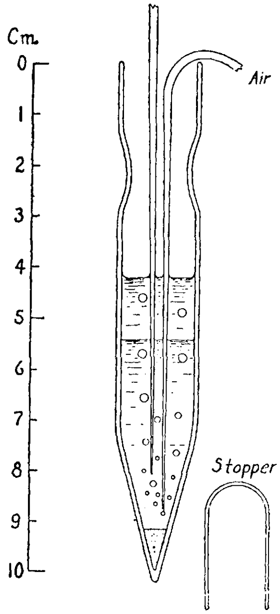
[*] Sodium and potassium thiocyanates frequently contain traces of chloride: this is never the case with the ammonium salt.

[†] A double size with a capacity of 0·2 c.c., subdivided into c.mm., has recently been constructed in this laboratory.

is centrifuged for 10—15 minutes at 3000 R.P.M., three drops of saturated ferric alum [$Fe_2(SO_4)_3,(NH_4)_2SO_4,24H_2O$] solution, followed by about 2 c.c. of ether, are added and titration is made with 0·1*M*-ammonium thiocyanate delivered from the Rehberg microburette.

Fig. 1 gives a diagrammatic representation of the appearance of the tube during the titration. The very fine delivery tip of the microburette dips directly into the titration mixture and stirring is accomplished by a current of washed air or nitrogen from a second fine glass tube reaching to within 3—4 mm. of the top of the silver chloride precipitate. As a result of centrifuging, the precipitate forms a compact mass which offers very little exposed surface and is almost completely removed from the sphere of titration.

FIG. 1.



Titration is carried out at a rate such that the silver thiocyanate precipitate is almost entirely flocked and carried to the interface between the ether and the titration mixture shortly before the end-point is reached. The end-point is taken as the first definite trace of salmon-pink which persists for more than 5 seconds. The colourless ethereal layer and a milk-glass plate behind the titration tube afford excellent conditions for comparison. To achieve the highest accuracy, in which the end-point is judged within 0·1—0·2 c.mm. of the thiocyanate, requires some practice in judging the rate of titration and the exact end-point, but no unusual ability is necessary. After the burette is read, an additional c.mm. is added to satisfy the analyst that the end-point was correct. Titration is carried out by white light; excellent results are obtained with a " daylight " lamp at night or in a dark room.

The calculation of the results may be made in a variety of ways. The basic reaction, in milli-equivalent terms, c.c. $AgNO_3[AgNO_3]$ = c.c. $Cl^-[Cl^-]$ + c.c. $NH_4CNS$ [$NH_4CNS$], may be used in the following form: mg. chloride/100 c.c. =

$$\frac{10000\ (\text{c.c. } AgNO_3)[AgNO_3]}{2{\cdot}8203\ (\text{c.c. } Cl^-)} - \frac{10\ (\text{c.mm. } NH_4CNS)[NH_4CNS]}{2{\cdot}8203\ (\text{c.c. } Cl^-)}.$$

Since only 1—2% of the total silver nitrate is titrated with thiocyanate, it is not necessary to know the titre of the ammonium thiocyanate with extreme precision; 1%, or at most 0·5%, accuracy will suffice.

Two different known amounts of chloride may be used for the determination of the various concentrations. This is particularly convenient when the strength of the thiocyanate is not known. Two standard chloride solutions may be used, but it is easier to use two different amounts of the same standard solution in view of the ease of adjustment of the syringe-pipettes.

Fig. 2.

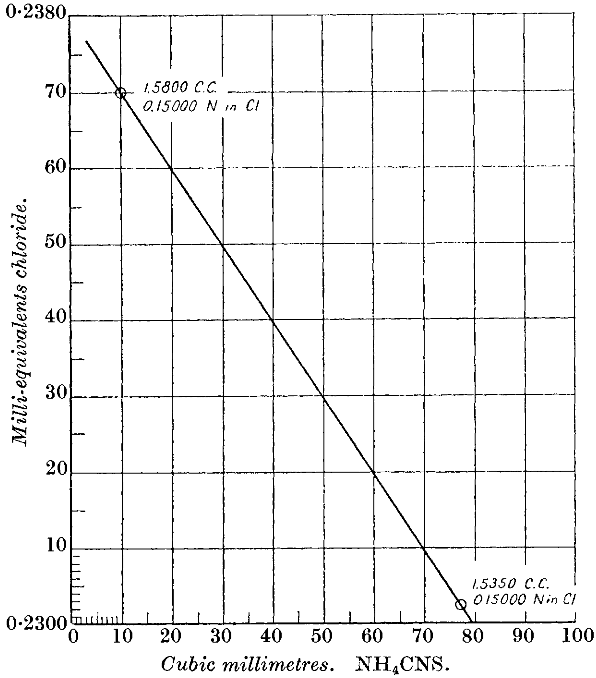


Fig. 2 shows a typical graph of two known amounts of chloride, from which the milli-equivalents of chloride in the unknowns may be read off directly.

*Scheme for Routine Chloride Determinations.*—Approximate chloride concentrations, where not known, are determined within roughly 1% by any of the numerous rapid methods which are available. The Rehberg micro-method (*Biochem. J.*, 1926, **20**, 483) requires only 0·1 c.c. and is very rapid. On the basis of the approximate chloride concentrations, appropriate syringe-pipettes and suitable silver nitrate concentrations are chosen. The various samples and solutions are allowed to come to the same temperature.

The chloride unknowns and standards are delivered into the centrifuge titration tubes, the syringe-pipette being flushed out several times whenever a new solution is taken. Two or more tubes are used for each different solution, but the syringe-pipette is not flushed between these deliveries. The silver nitrate solution is now delivered into the tubes in lots of 5 to 10 tubes at a time. When the addition of silver nitrate is completed for a set of tubes, they are shaken, the sides washed down with the nitric acid, the glass capsules inserted in the mouths, and the lot placed in a dark room or light-tight cupboard. Several standard chlorides are usually included in about each fourth lot of tubes.

On the following day the tubes are centrifuged, and returned to the dark to be withdrawn in sets of 5 or 6 for titration : the ferric alum and ether are added immediately prior to titration. In the meantime the microburette has been flushed out four or five times with the ammonium thiocyanate to be used, and both the burette tip and the air delivery tube wiped off with water. After the completion of each titration these tubes are wiped off with dry filter-paper, two more drops of the thiocyanate solution delivered, the burette tip again wiped off, and the burette re-filled. The silver nitrate is protected from unnecessary exposure to the light at all times.

A large number of observations have shown that when the amount of thiocyanate needed for titration is increased much beyond 0·005 milli-equivalent, there is a considerable loss of accuracy with the method. Adherence to this titration limit imposes some difficulty when solutions more concentrated than 800 mg. chloride/100 c.c. are encountered and for these cases we find it convenient to dilute the chloride samples before the final analysis. For example, sea water of salinity 35% may be diluted with 2 parts of water.

*Results obtained with the Present Method.*—The method which has been described enables the analyst to perform 60—70 analyses a day and uses only 1 to 2 c.c. in the actual determinations. With a 10 c.c. sample, the approximate chloride may be determined and an exact analysis made in triplicate. The percentage accuracy is related to the chloride concentration, but with concentrations of the order of 600 mg. Cl/100 c.c. it is not difficult to distinguish between solutions differing by only 1 part in 10,000 of chloride.

The following table gives the results of over 100 analyses made on 24 different solutions of four types. The figures are really for total halide reckoned as chloride; as in practically all other chloride methods, there is no distinction made between chloride, bromide, and iodide.

*Results of Chloride Determinations* (*g. chloride*/1000 c.c.).

Solutions of NaCl and KCl.

| | | | | | | |
|---|---|---|---|---|---|---|
| | 5·7836 | 5·7498 | 5·7095 | 5·7261 | 5·6952 | 6·1595 |
| | 5·7830 | 5·7501 | 5·7092 | 5·7258 | 5·6952 | 6·1602 |
| | 5·7833 | 5·7486 | 5·7086 | 5·7272 | 5·6952 | 6·1600 |
| | 5·7827 | 5·7506 | — | 5·7272 | — | 6·1602 |
| | 5·7836 | 5·7488 | — | — | — | 6·1600 |
| Mean | 5·78324 | 5·74968 | 5·7091 | 5·7266 | 5·6952 | 6·1600 |

Solutions containing $Cl^-$, $Na^+$, $K^+$, $Ca^{++}$, $HCO_3^-$, $SO_4^{--}$.

| | | | | | | |
|---|---|---|---|---|---|---|
| | 5·7040 | 5·7191 | 5·7486 | 5·7072 | 6·1804 | 5·0381 |
| | 5·7037 | 5·7188 | 5·7515 | 5·7069 | 6·1801 | 5·0387 |
| | 5·7037 | 5·7188 | 5·7486 | 5·7076 | 6·1807 | 5·0379 |
| | 5·7019 | 5·7182 | 5·7486 | 5·7075 | 6·1804 | 5·0376 |
| | — | — | — | 5·7073 | 6·1801 | 5·0379 |
| Mean | 5·7033 | 5·7187 | 5·7493 | 5·7073 | 6·1803 | 5·0380 |

Physiological perfusion media containing $Cl^-$, $Na^+$, $Ca^{++}$, $HCO_3^-$, urea, dextrose.

| | | | | | | |
|---|---|---|---|---|---|---|
| | 6·1353 | 6·1758 | 6·9598 | 6·1210 | 6·1299 | 6·9594 |
| | 6·1355 | 6·1748 | 6·9587 | 6·1210 | 6·1299 | 6·9596 |
| | 6·1355 | 6·1758 | 6·9607 | 6·1212 | 6·1297 | 6·9592 |
| | 6·1351 | 6·1754 | 6·9594 | — | 6·1301 | 6·9592 |
| | — | 6·1760 | 6·9596 | — | 6·1295 | 6·9598 |
| Mean | 6·1353 | 6·1756 | 6·9596 | 6·1211 | 6·1298 | 6·9594 |

Sea water (with high organic and nitrogen content) in various concentrations.

| | | | | | | |
|---|---|---|---|---|---|---|
| | 6·3550 | 6·3250 | 18·9716 | 18·8754 | 18·9750 | 19·0650 |
| | 6·3558 | 6·3250 | 18·9704 | 18·8761 | 18·9757 | 19·0643 |
| | 6·3539 | 6·3252 | 18·9716 | 18·8754 | 18·9750 | 19·0631 |
| | 6·3548 | 6·3250 | — | 18·8750 | 18·9750 | 19·0672 |
| Mean | 6·3549 | 6·3251 | 18·9712 | 18·8755 | 18·9752 | 19·0649 |

The method has been applied to routine determinations of chloride in this laboratory over a period of some months; in over 700 analyses the average variation between duplicates has been about 1 part in 5000 of chloride.

### *References.*

Alefeld, E.: Eine Vereinfachung der gravimetrischen Halogensilberbestimmung (*Z. anal. Chem.*, 1909, **48**, 78).

Bang, Ivar: Ein Verfahren zur Mikrobestimmung von Blutbestandteilen (*Biochem. Z.*, 1913, **49**, 19).

Buday, L.: Bestimmung der Chloride im Harn und im Blut mit Hilfe der Leitfähigkeitstitration (*ibid.*, 1928, **200**, 166).

Claudius, M.: A new method for the micro-determination of chlorine in blood and other albuminiferous media (*Acta Med. Scand.*, 1924, **61**, 4).

Christy, R. K., and W. Robson: The determination of chloride in biological fluids (*Biochem. J.*, 1928, **22**, 571).

Drechsel, E. (*J. pr. Chem.*, 1877, **15**, 191; quoted by H. Fresenius, "Berichte über die Fortschritte der analytische Chemie," pp. 351—353; *Z. anal. Chem.*, 1877, **16**, 341).

Duboux, M., and L. Parchet: Microdosage des chlorures dans le sérum et le liquide céphalorachidien par la methode des conductibilités électriques (*Bull. Soc. Chim. biol.*, 1929, **11**, 504).

Eisenmann, A. J.: A note on the Van Slyke method for the determination of chlorides in blood and tissue (*J. Biol. Chem.*, 1929, **82**, 411).

Fajans, K., and Hassel (*Z. Elektrochem.*, 1923, **29**, 495).

Földes, E., and H. Tauber : A new method for the determination of chlorides in the blood (*J. Lab. Clin. Med.*, 1929, **15**, 59).

Forbes, J. C., and H. Irving : An electrometric method for the determination of chlorides in whole blood and animal tissues (*J. Biol. Chem.*, 1929, **83**, 337).

Harvey, S. C. : The quantitative determination of the chlorides in the urine (*Arch. Intern. Med.*, 1910, **6**, 12).

Heidlberg, T. von : Nephelometrische Bestimmung von Chlor und Silber (*Biochem. Z.*, 1928, **192**, 238).

Hoitsema, C. (1904) : Ueber eine angebliche Änderung der Volhardschen Silberbestimmung (*Z. angew. Chem.*, 1904, **17**, 647).

Kolthoff, I. M., W. M. Lauer, and C. J. Sunde : The use of dichlorofluorescein as an adsorption indicator for the argentometric titration of chlorides (*J. Amer. Chem. Soc.*, 1929, **51**, 3273).

Langelaan, J. W. : Some observations on the silver chloride electrode in contact with Ringer's solution (*Arch. Néerland. Physiol.*, 1929, **14**, 592).

Laudat, M. : Le dosage du chlore dans le sérum sanguin et dans les liquides albumineux de l'organisme (*J. Pharm. Chim.*, 1917, **16**, 168).

Lewis, R. C., and N. L. Binkley : A method for the determination of blood chlorides (*J. Biol. Chem.*, 1930, **87**, 23).

McLean, F. C., and D. D. Van Slyke (*J. Amer. Chem. Soc.*, 1915, **37**, 1128).

McLean, F. C., and D. D. Van Slyke : A method for the determination of chlorides in small amounts of body fluids (*J. Biol. Chem.*, 1915, **21**, 361).

Muller, E. : Zur electometrischen Bestimmung der Chloride (*Z. Elektrochem.*, 1924, **30**, 420).

Oxner, M., and M. Knudsen : Manuel pratique de l'analyse de l'eau de mer. I. Chloruration par la methode de Knudsen (Bull. Comm. Internat. pour l'Exploration de la Mer Méditerranée, No. 3, April 1920, Monaco).

Prikladowizky, S., und A. Apollonow : Jodometrische Mikromethode zur Bestimmung der Chloride (*Biochem. Z.*, 1928, **200**, 135).

Rosanoff, M. A., and Arthur E. Hill : A necessary modification of Volhard's method for the determination of chlorides (*J. Amer. Chem. Soc.*, 1907, **29**, 269).

Rose, W. B., and C. J. Stucky : A micro-method for the estimation of chlorides in blood (*Mikrochemie*, Pregl Festschrift, 1929, 300).

Rothmund, V., and A. Burgstaller : Ueber die Genauigkeit der Chlorbestimmung nach Volhard (*Z. anorg. Chem.*, 1909, **63**, 330).

Smirk, F. H. : The micro-estimation of chlorine in whole blood, serum, or corpuscles (*Biochem. J.*, 1927, **21**, 31).

Sutton, F. ("Volumetric Analysis," 1924, 11th ed., J. and A. Churchill, London).

Sørensen, S. P. L. : Berichte über die Konstantenbestimmungen zur Aufstellung der hydrographischen Tabellen (Martin Knudsen). III. Chlor- und Salzbestimmung (*D. Kgl. Danske Vidensk. Selsk. Skrifter*, 1902, **12**, 93).

Tillmann und O. Heublins (*Chem. Ztg.*, 1913, 901).

Treadwell, F. P. ("Kurzes Lehrbuch der analytischen Chemie," 1913, **2**, 1te Aufl. Deuticke, Leipzig und Wien).

Tschopp, E. : Eine Mikromethode zur Bestimmung der Chloride in biologischen Flüssigkeiten und Organen (*Mikrochemie*, 1927, **5**, 161).

Van Slyke, D.D. : The determination of chlorides in blood and tissues (*J. Biol. Chem.*, 1923, **58**, 523).

Volhard, J. (*Sitzungsber. Acad. Wiss. München*, 1874, 1; *J. pr. Chem.*, 1874, **9**, 217).
Volhard, J.: Die Anwendung des Schwefelcyanammoniums in der Massanalyse (*Annalen*, 1878, **190**, 1).
Whitehorn, J. C.: A system of blood analysis. Suppl. II. Simplified method for the determination of chlorides in blood or plasma (*J. Biol. Chem.*, 1920, **45**, 449).
Wilson, D. Wright, and Eric G. Ball: A study of the estimation of chloride in blood and serum (*ibid.*, 1928, **79**, 221).
Winkler, L. W.: Ueber die Bestimmung des Chlor-Ions in natürlichen Wassern (*Z. anal. Chem.*, 1914, **53**, 359).

## *Summary.*

A rapid method for the determination of chlorides utilising the Volhard titration is presented. The method uses 1—2 c.c. of chloride solution, and determinations in triplicate may be made on a sample of 10 c.c. The absolute sensitivity is of the order of 0·001 mg. chloride.

A scheme for routine chloride determinations is given; following this procedure, one analyst can make over 50 analyses per day.

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