method for calculating activities in such solutions has been presented. The activity of sulfuric acid has been found to vary regularly with change in the concentration of glycol present. Abnormalities in the inversion of sucrose in the presence of ethylene glycol, as catalyzed by sulfuric acid, cannot be attributed to abnormal variations in the activity of the acid in the presence of glycol.

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A Rapid Micro Method for the Determination of Chlorides in Fluids

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The following method of chloride determination was devised especially for the analysis of very small amounts of fluids and in particular those of biological importance. Certain body fluids, such as peripheral lymph, or the blood of very small animals, or of insects, can be obtained only in such small quantities that analyses hitherto have been very difficult or out of the question.

A necessary step in the volumetric determination of chlorides by means of silver nitrate is that of exactly estimating the excess of silver used in precipitation. Field[1] in 1860 employed iodine dissolved in potassium iodide solution using starch for the end-point, having first neutralized the excess of nitric acid in the solution. King[2] reduced the amount of nitric acid (which interferes with the starch end-point), filtered the silver chloride suspension and titrated the filtrate with potassium iodide solution, using sodium nitrite to oxidize the excess of iodide. McLean and Van Slyke[3] using the same procedure considerably sharpened the end-point by the introduction of a buffer salt—sodium citrate. In the iodimetric method, as in the Volhard method, it is necessary to filter the excess of silver nitrate from the precipitated silver chloride, before completing the titration with potassium iodide; otherwise the latter reacts with the solid silver chloride. For very exact work it has also been shown[4] that it is necessary to remove the precipitated silver iodide just before the end-point is reached, as otherwise the latter is obscured.

The filtration and washing are not only time-consuming but so dilute the sample that for very small amounts of chloride the accuracy is diminished. This step has been eliminated in the following method by carrying out the

(1) Field, *Chem. News*, **2**, 17 (1860).
(2) King, *Merck's Rept.*, **18**, 57 (1909).
(3) McLean and Van Slyke, THIS JOURNAL, **37**, 1128 (1915).
(4) Lamb and Fairhall, *ibid.*, **45**, 390 (1923).

entire analysis in a single 15-cc. centrifuge tube and centrifuging out the silver chloride and the silver iodide at the appropriate points. The silver halide forms a hard, compact mass at the tip of the centrifuge tube, presenting but little surface, and gives no evidence of interference with the end-point in periods of time much in excess of that required for completion of the titration. Therefore no extra manipulation or washing is required and the analysis may be carried to completion in the one tube without transference.

Exact volume measurements of the small amounts of fluid or of the solutions used constitute a necessary step in the procedure and were made by means of extremely fine bore capillary pipets made from thermometer tubing of 0.3–0.5 mm. internal diameter. These pipets are easily made by blowing a bulb in the middle of a convenient length of this tubing and calibrating between two points. One end of the tube should be drawn out to a fine point. By using a fine tip the usual tendency to wet the outside of the tube by the creeping back of liquid was avoided. Typical sampling pipets had volumes of 0.0738 cc. and 0.1106 cc. The silver nitrate solution was also measured from a special pipet of about 1-cc. volume which was provided with a three-way capillary stopcock and a reservoir. A calibrated microburet of 5-cc. capacity was used for titration with the potassium iodide solution.

Fluids containing protein may be directly analyzed for chloride content by first precipitating the protein with tungstic acid and centrifuging. The usual reagents are then added to the supernatant fluid without disturbing the precipitate. This procedure considerably simplifies the analysis of such fluids as blood and lymph.

Method of Analysis

The method finally adopted is as follows. To a 15-cc. centrifuge tube add about 0.5 cc. of water and introduce the sample (about 0.1 cc.) using the accurately calibrated sampling pipet for this purpose. Wash the sides of the tube with a little water and stir well with a small stirring rod, which is left in the tube. Add 1 cc. of tungstic acid protein precipitant (when the tungstic acid reagent becomes cloudy it must be discarded) and stir thoroughly. Remove the stirring rod and rinse with a few drops of water. Again wash the sides of the tube with water and centrifuge at high speed for three or four minutes. The precipitated proteins will be thrown down as a firm mass in the case of serum or blood, leaving a water-clear supernatant liquid. Add an accurately measured excess of silver nitrate solution (about 1.5 cc. of silver nitrate solution, 1 cc. of which is equivalent to 1 mg. of sodium chloride and which contains 250 cc. of nitric acid per liter of solution). Shake the contents of the tube gently to ensure thorough mixing and centrifuge at high speed for two or three minutes. The silver chloride will be thrown onto the protein precipitate as a fine, compact layer. From the microburet add standardized potassium iodide solution (half the equivalent strength of the silver nitrate solution) to within a few drops of the end-point. Thoroughly mix and add 1 cc. of nitrite–citrate buffer (20 g. of sodium nitrite and 446 g. of sodium citrate per liter of solution) and starch paste. Centrifuge for a minute or so at high speed. The silver iodide will be thrown out, leaving a clear supernatant liquid. Unless the bulk of silver

iodide is so removed at this point, it obscures the end-point. Continue the titration to that point at which the first faint permanent color appears when looking down through the tube. The tube may be stoppered and gently inverted to ensure thorough mixing at this last stage of titration without disturbing the precipitate. While in the centrifuge it is well to keep the tubes capped on all occasions in order to prevent contamination with chloride bearing dust. Once the solutions are accurately adjusted and the apparatus calibrated, the actual analysis of blood serum or of lymph requires but fifteen or twenty minutes.

Determinations of known amounts of pure potassium chloride by this method and comparison with the similar macro volumetric method involving filtration and washing gave excellent results. Analyses were also made of serum, of whole blood and of serum to which known amounts of potassium chloride had been added. These results are shown in Table I.

TABLE I

SUMMARY OF ANALYSES

Material	Normality of $AgNO_3$ soln.	No. of analyses	Cl present, mg.	Max. error, %	Av. error, %	Av. deviation, %
Pure KCl	0.03418	20	0.71–0.45	−2.7	−0.07	0.74
Pure KCl	.01709	4	0.30	−2.7	−2.1	.60
Serum	.01709	7	0.31	−2.3	−0.6	.15
Serum + 0.3 mg. KCl	.01709	10	0.61	−2.8	−1.8	1.4
Whole blood	.01709	13	0.31–0.37	−4.5	−1.3	2.5

It is apparent that in 20 determinations of pure potassium chloride ranging in amount from 0.71 to 0.45 mg. the average error was −0.07% with a maximum error of 2.7%, while in four determinations of a smaller amount of potassium chloride (0.30 mg.) the average error was −2.1%, with a maximum error of −2.7%. With serum, serum containing known added amounts of potassium chloride and with whole blood, the average error varied from −0.6 to −1.8%, the maximum error being −4.5% in the case of one of the whole blood analyses. The average error of all the analyses was −1.3%.

Summary

A rapid micro method for the determination of the chloride content of biological or other fluids is described. With this method it is possible to determine a fraction of a milligram of chloride in 0.1 cc. of solution with an accuracy of about 2%.

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