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## A Convenient Method for Determining Chlorides in Urine: Modification of Northrop's Potentiometric Titration.* (19868)

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(Introduced by E. M. Landis.)
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To meet a need for rapidly determining the chloride concentration in a large number of small specimens of urine and other biological fluids the method of potentiometric titration recently described by Northrop(1) was selected because of its simplicity and precision. The great convenience of this method was confirmed; but the values obtained with urine were found to be in error because of interference by urates. Modified solutions eliminating such interference are being described because 4 years' experience has shown that the method as modified (a) is much faster than other methods in common use once the equipment has been assembled, (b) is quite suitable for small samples, and (c) provides a much more satisfactory end-point, facilitating greater precision even in the hands of a beginner.

*Apparatus and methods.* The method, based on the well-established principles of potentiometric titration(2), consists of the titration of the chloride with a standard solution of silver nitrate in the presence of an appropriate electrolyte, using the voltage of a silver electrode to indicate the end-point.

The diagram of the apparatus shown in Fig. 1, modified from Northrop(1), specifies the composition of the solutions in terms of their final molarity, the number of significant

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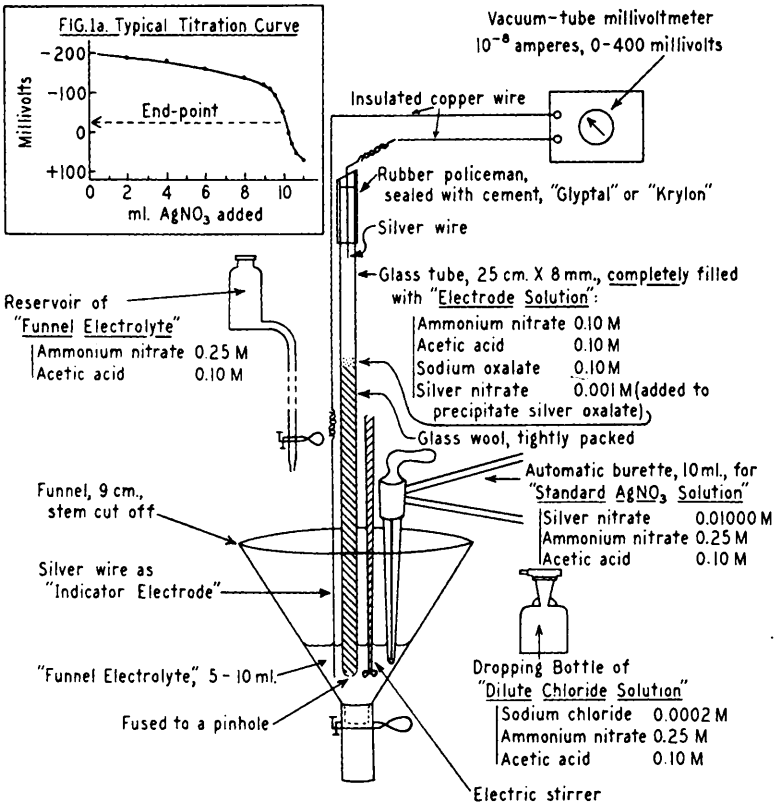


FIG. 1. Arrangement of apparatus and composition of solutions.
FIG. 1a (Inset). Typical titration curve, from which meter reading at end-point is determined.

figures indicating the requisite accuracy. The essential modification is the use of ammonium nitrate and acetic acid to maintain an acid pH while keeping liquid junction potentials insignificant. The "Standard $AgNO_3$ Solution" is accurately prepared from pure dried $AgNO_3$. It is stable for months when protected from light. The "Dilute Chloride Solution" is conveniently prepared by adding a drop of isotonic saline to about 50 ml of "Funnel Electrolyte." The reference or oxalate half-cell, formed by the silver wire in the tube of "Electrode Solution," is stable indefinitely if its lower tip is not allowed to dry. We have had to refill this tube with "Electrode Solution" twice in 4 years, because of leaks. A titration curve is performed when a new oxalate half-cell is prepared: plotting voltmeter readings in arbitrary units against ml $AgNO_3$ added (Fig. 1a). Theoretically, the steepest point of this curve is the equivalence point. (Actually, the chloride or indicator electrode is then about 20 to 50 millivolts negative to the oxalate electrode.) As this point is quite constant for any given oxalate half-cell, this meter reading (without calibration to convert into millivolts) is used as the end-point throughout the life of the half-cell (years). The titration curve can be repeated if doubt ever arises as to the condition of either electrode.

Since, at the end-point, the voltage between the electrodes is not too far from zero, and is changing at the rate of 7 to 40 millivolts per micro-equivalent (0.1 ml) of $AgNO_3$ added (depending mainly upon the amount of fluid and protein in the funnel), and since the same meter reading is used at the beginning and end of each titration, analytical results are very insensitive to large percentage errors in the actual voltage selected as end-point. An accurate potentiometric instrument is therefore quite unnecessary. A very simple, conventional, vacuum-tube millivoltmeter of a continuously indicating type was substituted

TABLE I. Comparison of Determinations by Potentiometric Titration with Values Obtained by Other Methods.

| Nature of unknown | Spec. No. | Ref.* | Chloride conc. of specimen (mEq/L) Mean ± stand. dev. (No. of titrations) Ref. method | Potentiometric | Diff. | Aliquot size (ml) |
|---|---|---|---|---|---|---|
| Pure NaCl | A | I | 50 | 49.7 ± .1 (2) | — .3 | .5 |
| | A | I | 50 | 50 ± .4 (4) | 0 | 1 |
| | B | I | 100 | 99.8 ± .6 (3) | — .2 | .2 |
| | C | I | 100 | 99.8 ± .5 (5) | — .2 | .5 |
| | D | I | 160 | 159.5 ± .3 (3) | — .5 | .5 |
| | D | I | 160 | 160.2 ± .1 (4) | + .2 | 1 |
| | E | I | 200 | 201.3 ± .8 (3) | +1.3 | .2 |
| | F | I | 200 | 198.9 ± .3 (4) | —1.1 | .5 |
| Rat urine | A | IV | 74 ± .2 (4) | 73.3 ± .0 (5) | — .7 | .5 |
| | B | IV | 99 ± .6 (6) | 98.7 ± .2 (5) | — .3 | .5 |
| | C | IV | 139.2 ± .5 (7) | 139.5 ± .1 (5) | + .3 | .5 |
| Human urine | A | IV | 60.8 ± .3 (5) | 60.4 ± .1 (5) | — .4 | .5 |
| | B | IV | 108.6 ± .4 (5) | 108 ± .1 (5) | — .6 | .5 |
| | C | IV | 140.7 ± .3 (5) | 140.3 ± .2 (5) | — .4 | .5 |
| 80-85% evaporated milk sol. with NaCl added | A | IIIa | 110.3 ±2 (3) | | +4.8 | |
| | A | IIIb | 112.4 ± .3 (4) | 115.1 ± .9 (4) | +2.7 | .5 |
| | A | II | 115.5 ± .9 (3) | | — .4 | |
| | B | IIIa | 111.4 ±1.4 (3) | | +2.8 | |
| | B | II | 112.9 ± .6 (4) | 114.2 ± .7 (4) | +1.3 | .5 |
| | B | IIIb | 113.4 ± .4 (3) | | + .8 | |
| | C | IV | 104.2 ± .1 (5) | | +2.5 | |
| | C | V | 108.1 ±1.2 (6) | 106.7 ± .3 (5) | —1.4 | .5 |
| Beef serum | A | II | 98.7 ± .2 (4) | | +2.4 | |
| | A | IV | 98.9 ± .6 (6) | 101.1 ± .4(17) | +2.2 | .5, 1 |
| | A | VI | | 99 ± .3 (5) | +2.1 | 1 |
| | A | VII | | 98.5 ± .6(10) | +2.6 | 1 |

* Reference method used:

I Accurate preparation.
II Open Carius method of Van Slyke & Sendroy, "Macro" modification(3).
III " " " " " " " "Micro" " (3).
(a) performed by WRB, (b) performed by RHK with attention to details stressed by Eisenman(5).
IV Open Carius method of Wilson & Ball(4).
V Volhard method modified especially for milk, Method 1 of Sanders(7).
VI Analysis of 1:10 dilution by potentiometric method.
VII " " 1:10 " , deproteinized by tungstate method of Wu(13).

for a sensitive galvanometer because of its cheapness, ruggedness, and lower current drain, so that one or 2 cm of silver wire provide enough electrode surface. Such an instrument can be purchased; or a suitable one can be easily assembled from standard radio parts(2,pp.82-84).

*Procedure for analysis.* In preparation for a series of analyses, a large excess of chloride is added to the solution in the funnel. $AgNO_3$ is then added from the burette until the meter indicates the end-point. Since the solution in the funnel is now exactly neutralized, and its exact volume is immaterial (although small volumes increase sensitivity), analyses can begin. (a) The funnel is drained until 5 to 10 ml remain. (b) An aliquot (0.1 to 1.0 ml) of the specimen is pipetted into the funnel. (The neutralized solution already in the funnel facilitates rinsing of micropipettes calibrated "to contain.") (c) $AgNO_3$ is added from the burette until the meter again indicates the end-point. (d) The burette reading is mentally converted to the chloride concentration of the unknown in mEq/L by shifting the decimal point (and multiplying or dividing by 2 if necessary). Steps (a) to (d) are repeated for each succeeding analysis. If the end-point is exceeded, "Dilute Chloride Solution" can be added dropwise to neutralize the

TABLE II.
Results of Dilution of Unknown Solutions and Addition of Known Amounts of Chloride.

| Nature of unknown | Conc. of unknown in final sol., % (v/v) | Added chloride, mEq/L | Chloride conc. of final sol. Mean ± stand. dev. (No. of titrations) Calculated mEq/L | Observed, mEq/L | Diff., mEq/L | Aliquot size, ml |
|---|---|---|---|---|---|---|
| Rat urine "D" | 100 | 0 | * | 122 ± .7 (2) | | .2 |
| | 50 | 0 | 61 | 60.4 ± .5 (4) | —.6 | .2 |
| | 50 | 50 | 111 | 110.6 ± .5 (4) | —.4 | .2 |
| | 50 | 100 | 161 | 160.5 ±1 (4) | —.5 | .2 |
| | 20 | 0 | 24.4 | 23.5 ± .6 (4) | —.9 | .2 |
| Evaporated milk | 100 | 0 | * | 65.4 ± .5 (6) | | .5 |
| | 100 | 0 | * | 66.2 ±1.1 (4) | | 1 |
| | 80 | 80 | 132.6 | 131.7 ± .7 (3) | —.9 | 1 |
| | 40 | 160 | 186.3 | 186 ± .4 (2) | —.3 | 1 |
| | 4 | 160 | 162.6 | 162.7 ± .1 (2) | +.1 | 1 |
| | 1.2 | 80 | 80.8 | 80.6 ±0 (3) | —.2 | 1 |
| | 1.2 | 160 | 160.8 | 160.8 ± .1 (2) | .0 | 1 |
| Beef serum "A" | 100 | 0 | * | 101.1 ± .4 (17) | | .5, 1 |
| | 80 | 80 | 160.9 | 160.3 ± .7 (5) | —.6 | .5 |
| | 40 | 160 | 200.5 | 199.1 ±1 (5) | -1.4 | .5 |
| | 10 | 0 | 10.1 | 9.9 ±0 (5) | —.2 | 1 |
| | 4 | 160 | 164 | 163.7 ± .6 (5) | —.3 | .5 |
| | 1.2 | 80 | 81.2 | 81.2 ± .5 (5) | .0 | .5 |
| | 1.2 | 160 | 161.2 | 160.4 ± .6 (5) | —.8 | .5 |

* The other values in this column are calculated from the observed value in this row.

solution in the funnel before the titration is repeated. At the end of a series of analyses, accumulated precipitate is wiped off the glassware and indicator electrode; and the lower tip of the oxalate half-cell is capped by a rubber policeman, or simply reimmersed in fresh "Funnel Electrolyte," to prevent drying. Cleaning the indicator electrode occasionally with fine emery paper maintains its sensitivity.

*Validation of the method. Interfering substances.* Sufficient amounts of the following anions may be expected to interfere: bromide, chromate, cyanide, dichromate, ferricyanide, ferrocyanide, iodide, nitrite, thiocyanate and thiosulfate. Several mg or more of each of the following substances were added to the solution in the funnel at the end of a titration without producing any apparent effect on the end-point: acetone, ammonium carbonate, amytal, calcium nitrate, chloral hydrate, creatinine, dextran, ethyl alcohol, gelatin, glucose, inulin, lactic acid, paraldehyde, polyvinylpyrrolidone (PVP), sucrose, sulfathiazole, toluene, tryptophane, urea, urethane, uric acid and the following sodium salts: acetate, benzoate, bicarbonate, citrate, monobasic and dibasic phosphate, sulfate and tartrate.

*Pure NaCl.* The accuracy of the stoichiometric relationship is shown by titration of various volumes and concentrations of pure NaCl solutions (Table I). The mean values differ from the actual concentrations by less than 0.7% in each case.

*Urine.* Urine specimens from rats and man have been analyzed by standard procedures of the Volhard type(3-5), including hot digestion in the case of rat urine because of the protein normally present(6), and by this potentiometric method without any digestion (Table I). The results agree within 1%. Twenty-seven of these analyses were completed in an hour's time, except for the washing of the specimen containers. Analysis of a specimen of rat urine diluted various amounts, and with various amounts of NaCl and KCl added, showed satisfactory agreement with the concentrations calculated from the analysis of the original specimen (Table II).

*Evaporated milk.* When evaporated milk is added to the funnel electrolyte, a white floc appears in the clear liquid, and the titration can be performed without difficulty. Dilution and addition studies showed agreement within 0.7% (Table II). The "open Carius" method

of Van Slyke and Sendroy and some of its modifications(3-5), when applied to solutions of evaporated milk, gave results which commonly differed among themselves by 1 to 5% (Table I). Another modification of the Volhard method, designed especially for milk analysis(7, Method 1), also was distressingly inexact in our hands. The potentiometric method gave very consistent results, which differed from the reference values no more than the latter differed among themselves.

*Serum.* Upon the addition of serum, the solution in the funnel becomes opalescent, foam formation at the stirrer becomes troublesome, and the titration curve becomes much less steep. Added chloride was satisfactorily recovered (Table II), but the one sample of beef serum that has been studied carefully gave values 2% higher by direct potentiometric titration than by the other methods, which agreed closely among themselves (Table I).

*Discussion. Effect of protein.* Protein seems to interfere with some potentiometric chloride methods previously described(8), whereas its presence does not seem to impair the accuracy of some others(9). Probably these differences are due to the acidity of the solutions used (10,11) and the degree to which the protein is precipitated by the analytical conditions. In the present method, the small amount of protein in the urine of normal adult male rats (6) seems neither to precipitate, nor to cause error (Table I). Indeed, although large coagula of AgCl may form during the titration of purely inorganic solutions (impairing mixing), this is completely prevented by addition of a trace of gelatin to the solution in the funnel, without affecting the accuracy. In the analysis of milk, the prompt appearance of a white floc and the absence of frothing suggest that most of the protein is precipitated by the funnel electrolyte and causes no trouble. In the case of serum, the limited observations suggest that excessive amounts of protein remain in solution, causing the undesirable frothing and insensitivity, and combining with significant amounts of silver.

*Accuracy.* As an accuracy of 1% was considered adequate for the purposes for which this method was intended, commercial glassware was used without recalibration. In practice, considerably better precision has generally been obtained, and similar results can be expected in routine use. Indeed, precision comparable to the typical results presented in Tables I and II has been regularly achieved, after the first few hours' practice, by several other investigators who have adopted the method for their work. For urine, the absolute accuracy seems equally good. For milk preparations, the method is at least as accurate as the methods selected for comparison, and much more satisfactory. Although results tend to be higher than by the digestion procedures, the latter are known to be very susceptible to chloride loss(4,5,12). This is borne out by our experience that triplicate aliquots of a milk specimen digested simultaneously, although consistent, occasionally give very much lower values than other replications. The unsatisfactory nature of our initial experience with serum, compared with the satisfactory precision by other methods, discouraged us from further studies in this direction.

*Summary and conclusions.* 1. Modifications of Northrop's method of potentiometric titration of chloride are presented which retain its great convenience while eliminating interference from urates. 2. Analyses can be completed at the rate of 20 to 30 per hour, using aliquots of 0.1 to 1.0 ml, with a precision of better than 1%. 3. Accuracy of at least 1% for urine chloride determinations is evidenced by analysis of known solutions of NaCl, recovery of both KCl and NaCl added to urine specimens, and comparison of the values with those obtained by standard methods. 4. The method appears satisfactory for the analysis of evaporated milk preparations without modification: giving accurate recovery of added chloride, better consistency than reference methods, and as good agreement with them as their own variation (up to 5%) will allow. 5. The apparent unsuitability of the method for unmodified serum is discussed briefly.

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## Plasmal Reaction in the Blood and Hemopoietic Organs. (19869)

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Although the occurrence of the plasmal reaction of Feulgen and Voit(1) has been observed in many tissues and cells, no reference has been found in the literature concerning its presence in the cells of the blood and hemopoietic organs.

*Material and methods.* Ten adult normal rats of the Wistar strain were killed by a blow on the head. Smears of blood, bone-marrow, thymus, spleen and lymphatic glands were made and immediately immersed (before drying) in a mixture of equal parts of Schiff's reagent and saturated $HgCl_2$ solution during one minute, and then passed through 2 sulfurous acid baths of 5 minutes each and then rinsed with distilled water. Control smears were immersed in Schiff's reagent diluted with an equal part of distilled water for the same period, and afterwards treated as above described. Counterstaining with diluted methyl green solution was performed in some slides in order to help the identification of the cells. Smears from blood and sternal bone marrow obtained from 3 patients without hematological disorders were similarly treated. A positive reaction is indicated by a red stain present in the test slides and absent in the controls.

*Results.* As human and rat blood and bone marrow cells presented a similar reaction, the following data apply to both.

a) *Blood cells.* A positive reaction was observed in the cytoplasm of neutrophilic and eosinophilic granulocytes, monocytes and in the platelets. Reaction of neutrophils and monocytes was diffuse throughout the cytoplasm; eosinophils presented a less intense staining, localized between the generally unstained granules. Due to the small number of basophils no observations on them are reported. A positive reaction could be observed in the majority of the lymphocytes, sometimes diffuse throughout the cytoplasm and occasionally localized in granules. Red blood cells were always negative.

b) *Bone marrow cells.* The reaction was intensely positive in the cytoplasm of the

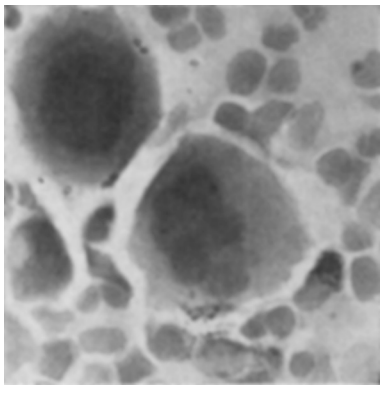

FIG. 1. Positive plasmal reaction in the cytoplasm of megakaryocytes and eosinophils. Nuclear counterstaining with methyl green. Approx. × 1400.