Ultramicro-colorimetry for Determination of Serum and Urine Chloride Using Silver Iodate

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INTRODUCTION

Mohr's silver nitrate-titration[1] using potassium chromate as the indicator is one of the oldest methods for chloride determination. Subsequently, various other methods were developed including titrations using thiocyanic-Fe[2] (rhodanic Fe) or diphenylcarbasone,[3] as indicators and colorimetry[4] using chloranilic acid mercury. With the recent development of polarography,[5] chloridimetry, and automation, rapid processing, greater specificity and reproducibility are possible.

At ABCC, serum Cl is determined routinely by a chloridimeter, which requires 0.1–0.2 mℓ. of serum. Occasionally, however, serum Cl is requested simultaneously with other electrolytes in adult patients or children from whom it is difficult to obtain a sufficient amount of blood for the determinations.

Therefore, review was made of the reagents and reaction conditions of the method of Hoffman et al.,[6] a modification of Sendroy's[7a-e] chloride titration that uses silver iodate in a colorimetric method, and a method was developed that required only 0.02 mℓ. of serum.

Values obtained by this method showed very good correspondence with those obtained by the chloridimeter and the macro-method of Hoffman et al.[6] This method also can be used for determinations of Cl in urine and spinal fluid.

THEORY

Protein is removed from the sample by precipitation with phosphoric tungstic acid. Silver iodate is added which reacts with chloride in the sample to produce sodium iodate and insoluble silver chloride:

$$AgIO_3 + NaCl \longrightarrow NaIO_3 + AgCl \qquad (1)$$

The precipitated proteins and silver iodide are removed by filtration (or

centrifugation) and the remaining solution, containing sodium iodate, is acidified with phosphoric acid and sodium iodide is added. For each equivalent of chloride in the original sample, 6 equivalents of free iodine are liberated:

$$NaIO_3 + 5NaI + 6H_3PO_4 \longrightarrow 3I_2 + 3H_2O + 6NaH_2PO_4 \quad (2)$$

The amount of free iodine (yellow) is measured spectrophotometrically, thus giving indirectly the amount of chloridde present.

REAGENTS

1) Phosphoric tungstic acid solution: Sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) 6 g. is put into a 1000 mℓ. flask, to which 0.15 M phosphoric acid is added to make a solution of 1000 mℓ.
2) 0.15 M phosphoric acid solution: 85 % phosphoric acid (H_3PO_4) 10 mℓ. is dissolved with H_2O to make a solution of 1000 mℓ.
3) Silver iodate ($AgIO_3$ Fisher Co. "Chloride free" or Wako SG) is used directly.
4) 0.4 % sodium iodide: 0.4 g. of sodium iodide (NaI) is measured and 0.1 N-NaOH 0.5 mℓ. is added. This is dissolved with H_2O to make a solution of 100 mℓ. (May be used for 2 to 3 weeks).
5) 120 mEq/ℓ NaCl standard solution: NaCl (special grade dried reagent) is redried for 3–5 hours at 100–110° C, immediately put into a desiccator and sealed. After it has cooled, 7.014 g. of this is measured and dissolved with H_2O to make a solution of 1000 mℓ. (May be used for a long period).
6) Standard working solution: 120 mEq/ℓ NaCl solution is diluted with H_2O to make working standards of 30, 60, 90 and 120 mEq/ℓ. These solutions may be stored in sealed glass containers for at least one month. When preparing the various concentrations, the solutions can be made easily and accurately by use of Sasaki's[8] standard solution dilution method.

Note: Use reagents with low Cl content, and all solutions are made using previously distilled water, filtered through an ion exchange resin.

PROCEDURES

A blank and 4 standards are prepared by dispensing into 5 tubes 0.02 mℓ (20λ) solutions containing respectively 0, 30, 60, 90 and 120 mEq/ℓ NaCl using a Sanz pipette. Unknowns containing 0.02 mℓ of serum are similarly prepared. To each tube, 0.5 mℓ of phosphoric tungstic acid are added and the contents thoroughly mixed. About 20 mg* of silver iodate are added to each tube,

*Exact weight unnecessary. The amount scooped onto the tip of a standard Japanese ear curette is sufficient.

thoroughly mixed, allowed to stand for about 5 minutes, and centrifuged at 3000 rpm for 5 minutes.

0.02 mℓ of the supernate of each centrifuged solution are transferred with a SANZ-pipette to corresponding test tubes a, b, c, d, e and f corresponding respectively to the blank, 4 standards and unknown, into which 3 mℓ of 0.4 % sodium iodide are added and, after standing for 10 minutes, spectrophotometry is performed using a filter of 420 mμ.

Using test tube "a" as a blank, the optical density of test tubes b, c, d, e and f is read; the optical desities of 30, 60, 90 and 120 mEq/ℓ solutions are used to construct a standard calibration curve, to determine, in turn, the Cl concentration of the unknown sample in tube f.

RESULTS

A comparison of chloride values in 75 serum samples, determined by the above method, the macro-method of Hoffman et al.[6] and chloridimetry is shown in Figure 1. The coefficients of correlation for the ultramicro method and chloridimetry is 0.991 and that for chloridimetry and the macro-method is 0.986.

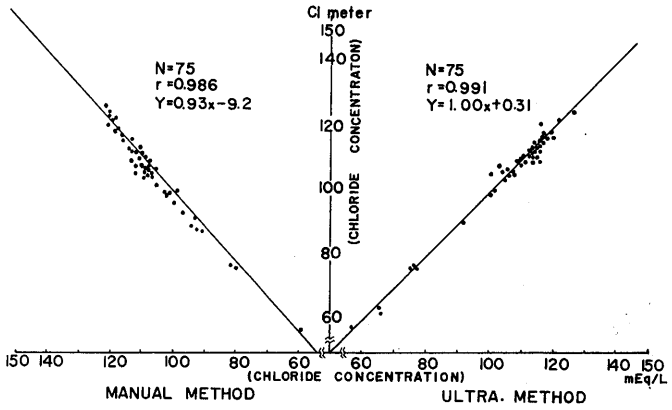

FIG. 1. COMPARISON OF SERUM CHLORIDE VALUES DETERMINED BY ULTRAMICRO METHOD, CHLORIDIMETRY AND MANUAL METHOD (mEq/ℓ)

Comparisons between the micro-method and chloridimetry, using 20 urine samples was performed and the correspondence was also satisfactory as shown in Table 1.

TABLE 1. COMPARISON OF CHLORIDE VALUES IN URINE DETERMINED BY ULTRAMICRO METHOD AND CHLORIDIMETRY

Sample No.	Determined Chloride Content (mEq/ℓ)		Difference (mEq/ℓ)
	Present Method	Chloride Meter	
1	182	180	+2
2	27	28	−1
3	178	174	+4
4	41	41	0
5	133	135	−2
6	120	123	−3
7	118	119	−1
8	172	172	0
9	73	77	−4
10	107	108	−1
11	169	170	−1
12	276	278	−2
13	249	248	+1
14	205	206	−1
15	93	91	+2
16	207	208	−1
17	266	269	−3
18	267	267	0
19	236	239	−3
20	237	238	−1

REVIEW

1. Absorption spectra and standard calibration curve

A Hitachi-Perkin-Elmer Type 139 spectrophotometer was used to determine the absorption spectra from 350 mμ to 590 mμ using a blank, and 50 and 100 mEq/ℓ NaCl at a slit width of 0.5 (Fig. 2). A maximum absorption peak could not be found within this range. For determinations in the ultraviolet areas, high-class colorimetric apparatus and reagents of high purity are required, making it susceptible to contamination, and therefore, it was decided that for routine tests spectrophotometric readings would be taken in the visible spectrum, specifically at 420 mμ.

Figure 3 shows standard calibration curves obtained using Gilford, Coleman Junior II, Coleman 6A and Coleman 6C colorimeters. The curves obtained with Gilford, and Coleman Junior II colorimeters were linear in accordance with Lambert-Beer's law, but the curves obtained with Coleman 6A and 6C colorimeters were slightly arc-shaped.

The deviation from linearity of the standard calibration curve for some of the

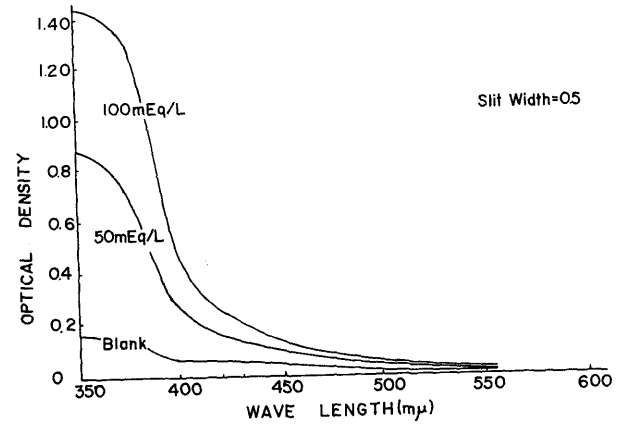

FIG. 2. ABSORPTION SPECTRA OF CHLORIDE SOLUTIONS
(By HITACHI-PERKIN-ELMER 139 UV-VIS Spetrophotometer)

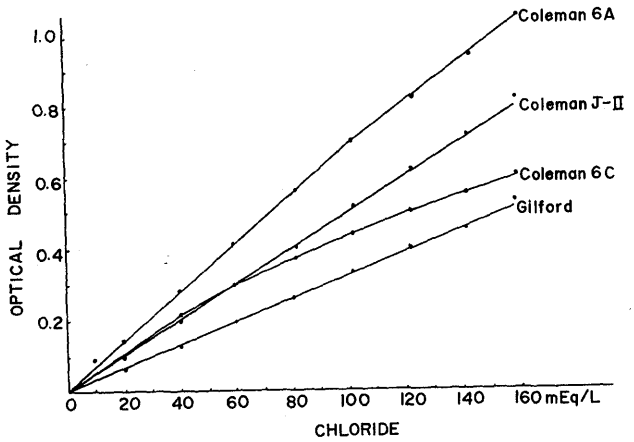

FIG. 3. STANDARD CALIBRATION CURVES FOR DIFFERENT SPECTROPHOTOMETERS

colorimeters emphasizes the importance of using at least 4 or 5 standard solutions of different concentrations to obtain a satisfactory curve. Dependence on a calibration curve derived from a standard solution of only a single concentration can lead to large errors in clinical chemical determinations. Further, standards and unknown samples should be processed in the same manner throughout the entire procedure.

2. Reagents

i) Sliver iodate: Concentration and commercial variation

The effect of increasing concentrations of silver iodate in the procedure (see

equation 1) was investigated by adding, to a series of standard test solutions containing 50 mEq/ℓ NaCl, 20 mg. increments of silver iodate in a range between 20–120 mgs. Commercially available iodate from three different manufactures, A, B and C were tested. Judging from the final color reaction (Fig. 4), the iodate from companies A and C precipitated a fixed amount of chloride irrespective of the amount of iodate added, but that of company B showed an increase in chloride proportional to the amount of iodate added, indicating considerable contamination with chloride rendering the reagent unfit for use in the method without further purification.

Comparison of the variation between different lots of silver iodate made by companies A and C showed no major differences. The products of these two companies could be used directly without further purification.

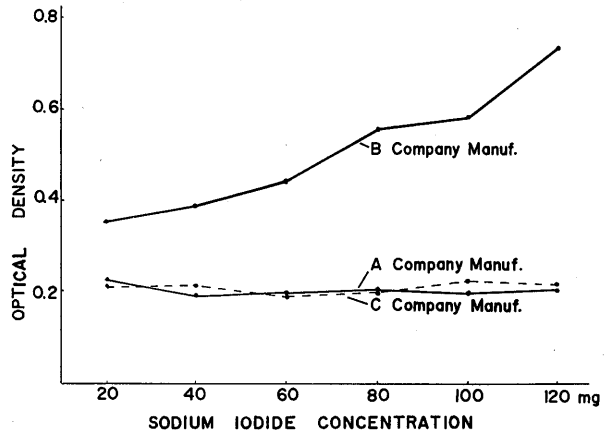

FIG. 4. EFFECT OF CONCENTRATION OF SODIUM IODIDE ON COLOR REACTION

ii) Sodium iodide: Concentration and volume

The effect of different concentrations of sodium iodide (see equation 2) on the color reaction was investigated. Standard test samples containing 100 mEq/ℓ NaCl were processed in the usual fashion (see procedure). To the final 0.02 mℓ supernates, sodium iodide (3 mℓ.) was added, in the following concentrations: 0.4, 0.6, 0.8 and 1.0 %. Over this range of reagent concentration, there were no differences in the optical densities of the test solutions, and the 0.4 % concentration was chosen for the procedure.

Review was made of the color reaction when 2, 3, 4 and 5 mℓ. of 0.4 % sodium iodide were added. The color intensity of the reaction decreased with increasing volume of reagent, which is obviously a dilution effect. Adequate color intensity was attained with 2 mℓ. of 0.4 % sodium iodide solution. Thus, the volume of sodium iodide may be adjusted according to the size of the colorimeter

cuvette. In this laboratory 3 mℓ. is a convenient volume to use.

3. Color reaction time and fading time

At 25° C, maximum color intensity developed immediately after addition of sodium iodide and remained stable for at least 90 minutes.

4. Effect of temperature

An incubator with an adjustable temperature control was used to test the effect of temperature at 10°, 20°, 30°, 35°, 40° and 50°C. Within this range, there was no significant effect on the Cl determinations.

5. Effect of some other substances in blood

Protein (human albumin 10 g/dℓ), hemoglobin (14 g/dℓ), bilirubin (20 mg/dℓ), glucose (100 mg/dℓ), urea N (100 mg/dℓ), vitamin C (100 mg/dℓ) and cholesterol (200 mg/dℓ) in amounts of 0.02 mℓ. respectively were added to standard test solutions containing 20, 40, 60, 80 and 100 mEq/ℓ NaCl, and Cl value was determined. No effects due to these substances could be detected.

6. Precision and accuracy

i) Repeatability: Twenty replicate determinations of the same serum were performed. The results were: Mean = 108.8 mEq/ℓ; S.D. = 0.61; C.V.* = 0.56 % indicating excellent repeatablility.

ii) Reproducibility: Determinations were performed over a period of 20 days using the same sample. Figure 5 shows the results. Each point in the upper

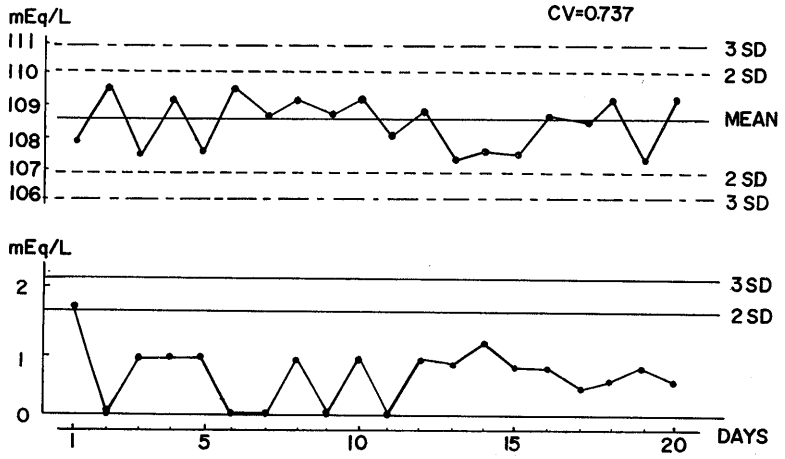

FIG. 5. REPRODUCIBILITY OF CHLORIDE DETERMINATIONS

Upper Panel: Each point is the average of duplicate determinations for that day.
Lower Panel: Each point is the difference between duplicates for that day.

*Coefficient of variation $= \frac{\text{S.D.}}{\text{Mean}} \times 100$

panel is the average of duplicate determinations for each day. Each point on the lower panel shows the difference in mEq/ℓ between the duplicates for that day. The overall mean for the 20 day period = 108.5 mEq/ℓ ; S.D. = 0.80 ; C.V.= 0.73 %. Reproducibility for this method is also excellent, though variation is slightly greater than under item 6.i) above.

iii) Recovery experiments : Table 2 shows the results obtained by adding known amounts of chloride to test solutions and comparing the calculated results to those obtained by actual determination of the chloride content. Column 2 (from the left) shows the amount of chloride. Column 3 gives the chloride content of a commercial preparation* which, when combined with an appropriate volume of the test solution from column 2, gives a calculated total chloride content shown in column 5. The chloride content of these artificial mixtures was determined and the results are shown in column 4. Finally, the percent 'recovery' (observed/expected or determined/calculated) is shown in column 6. The percent recovery was over 99 % for all but the two higher concentrations of chloride.

TABLE 2. COMPARISON OF CALCULATED AND DETERMIND CHLORIDE CONTENT OF VARIOUS TEST SOLUTIONS

Test Tube No.	Amount of Chloride Solution (mEq/ℓ)	Serum Chloride Known (mEq/ℓ)	Observed (mEq/ℓ)	Theoretical (mEq/ℓ)	Recovery (%)
1	10.2	30.2	40.2	40.4	99.5
2	20.0	30.2	49.8	50.2	99.2
3	39.9	30.2	70.0	70.1	99.8
4	50.1	30.2	80.2	80.3	99.8
5	78.1	30.2	105.3	108.3	97.2
6	96.8	30.2	122.1	127.0	96.1

SUMMARY

An ultramicro-colorimetric method was developed based on Hoffman's modification of Sendroy's iodine chloride titration method using silver iodate. This method was shown to be comparable to other methods for determination of serum chloride and can be performed with as little as 0.02 mℓ. of serum.

This method correlates well with chloridimetry (coefficient of correlation 0.991). Repeatability of 20 duplicate determinations was excellent (coefficient of variation= 0.56 %) ; reproducibility was excellent (coefficient of variation= 0.73%).

*Versatol : Warner-Chilcott Laboratories, Morris Plains, New Jersey, U.S.A.

REFERENCES

1) Shibata, S. & Kitamura, M.: *Routine clinicobiochemical determinations.* Nakayama Shoten, Tokyo (1964).
2) Volhard, J.: Die Silbertitrierung mit Schwefelammonium. *Z. Anal. Chem.*, **17**: 482, 1878, 1).
3) Schales, O. & Schales, S. S.: A simple and accurate method for the determination of chloride in biological fluids. *J. Biol. Chem.*, **140**: 879-884, 1941.
4) Genba, A. & Oda, K.: Chloride determination by chloranylic acid mercuric salt method. *Rinshobyori* (Clinicopathology), **10**: 389-392, 1963.
5) Doberenz, W. E., Iseri, L. T., Zak, B. & Boyle, A. J.: Polarographic estimation of chloride in blood serum. *Amer. J. Clin. Path.*, **22**: 1207-1212, 1952.
6) Hoffman, W. S.: *Photoelectric Clinical Chemistry*, New York (1941), p. 129.
7a) Sendroy,J.: Microdetermination of chloride in biological fluids, with solid silver iodate. I. Gasometric analysis. *J. Biol. Chem.*, **120**: 335-403, 1937.
7b) ibid. II. Titrimetric analysis. *loc. cit.* **120**: 405-417, 1937.
7c) ibid. III. Colorimetric analysis. *loc. cit.* **120**: 419-439, 1937.
7d) ibid. Photoelectric microdetermination of chloride in biological fluids, and of iodate and iodine in protein-free-solutions. *loc. cit.* **130**: 605-623, 1939.
7e) ibid. Note on the photoelectric microdetermination of chloride in biological fluids. *loc. cit.* **142**: 171-173, 1942.
8) Shibata, S. & Sasaki, M.: *Routine Clinicochemical Ultramicrocolorimetry*. Kinpo-do, Kyoto (1967).