A Direct Photometric Method for Chloride in Biological Fluids, Employing Mercuric Thiocyanate and Perchloric Acid

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The reagent described is 5.5 M in perchloric acid and 3.3 M in urea. It contains ferric ions, mercuric thiocyanate, mercuric ions (from mercuric perchlorate), and mercuric chloride. Serum dissolves directly in this reagent to yield a clear, reddish solution.

When chloride ions are added, they combine first with the free mercuric ions, and then with some of the mercuric ions from the mercuric thiocyanate. Liberated thiocyanate combines with ferric ions to yield red ferric thiocyanate. The color is much more intense in the presence of strong perchloric acid than in other aqueous acid mixtures. Its intensity can be regulated at will by changing the concentration of the ferric ion. The presence of mercuric chloride in the reagent improves linearity between absorbance and chloride concentration.

After the total absorbance is determined, compensation for absorbance by other substances is secured by adding mercuric ions to the photometer tube to reverse the color-producing reaction of chloride, reading the residual absorbance, and subtracting it from the total absorbance, to give a net absorbance produced by chloride alone.

In view of previous reports of success with mercuric thiocyanate methods for the direct photometric measurement of chloride concentration in water (6) and in concentrated hydrogen peroxide (1), efforts were made to adapt the method to analysis of serum. The chemical principle depends upon the reactions

\[ 2 \text{Cl}^- + \text{Hg(SCN)}_2 \Rightarrow \text{HgCl}_2 \text{ (very slightly ionized)} + 2 \text{SCN}^- \]  
(1)

\[ \text{SCN}^- + \text{Fe}^{+++} \Rightarrow \text{FeSCN}^{++} \text{ (red)}. \]  
(2)

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Two problems arose: (1) interference due to the insolubility of protein in the strong acid used (HClO₄), and (2) low molar extinction. The proteins of serum were made soluble by including urea in the reagent. Sensitivity was greatly increased by increasing the concentration of perchloric acid. Then, however, absorbance and concentration of chloride did not show a straight-line relationship. Linearity was attained by adding mercuric chloride to the reagent. Equation 1 shows that such addition would stabilize the tendency toward reaction to the left.

Material and Methods

Reagents

The following solutions are used to make the color reagent:

*Perchloric acid, 60% w/w, analytical grade ACS* This acid is approximately 9.19 M.

*Urea, 50% (w/w)* Dissolve 500 gm. of urea in 500 gm. of water. If this concentrated solution is not colorless and clear, treat it as follows: Mix total volume with 1 ml. of 10% (w/v) ZnSO₄, 7H₂O, followed by 1 ml. of approximately 0.5 N NaOH (prepared as for making a zinc hydroxide protein-free filtrate of blood). A flocculation appears. Cover a coarse-porosity sintered glass filter with washed asbestos fibers (analytical grade), and filter the solution with suction. If necessary, pour the first portion through again. The resulting solution is clear and colorless. Total volume resulting is about 875 ml.

*Ferric nitrate, 2 M* Dissolve 40.4 gm. of Fe(NO₃)₃·9H₂O in 20 ml. of water and dilute to 50 ml.

*Mercuric perchlorate, 0.2 M* In a small beaker weigh out 4.33 gm. of C.P. mercuric oxide (red). Add 10 ml. of 60% perchloric acid and warm to dissolve. Cool and dilute to 100 ml. with water.

*Mercuric perchlorate, 0.02 M* Dilute the 0.2 M solution with water.

*Potassium thiocyanate, 0.4 M* Dissolve 3.89 gm. of pure KSCN in water and dilute to 100 ml.

*Mercuric chloride, 0.1 M* Dissolve 2.72 gm. of HgCl₂ in water and dilute to 100 ml.

*Sodium chloride, 0.2000 M* Weigh out 11.6900 gm. of C.P. dry NaCl, dissolve in water, and dilute to 1 L. in a volumetric flask. From this stock solution prepare solutions containing, per liter, respectively 80, 100, and 120 mEq.

Procedure for Color Reagent

The term ‘‘color reagent’’ is used throughout this paper (occasionally with reinforcement: ‘‘standard’’ color reagent) to identify the reagent
with the following exact composition. If the composition differs from the following, a difference is indicated by the term "modified reagent."

Use a 1500-ml beaker with a nonmetallic mechanical stirrer. Add in the order indicated, with rapid stirring: Urea, 50% (w/w), 350 ml.; and HClO₄, 60% (w/w), 600 ml. Mix; cool to about 20°. Add Fe(NO₃)₃, 2 M, 7.5 ml.; KSCN, 0.4 M, 12.5 ml.; Hg(ClO₄)₂, 0.2 M in a buret; titrate the red solution promptly to an exact colorless endpoint; required volume is about 12.5 ml. Add Hg(ClO₄)₂, 0.02 M, 15.0 ml.; and HgCl₂, 0.1 M, 6.0 ml. The mixture yields 1 L. of solution. Do not refrigerate, but keep below 30°.

**Nature of Constituents**

Perchloric acid greatly intensifies the color of ferric thiocyanate in strong concentrations of acid. Other strong mineral acids, such as sulfuric, can be used, but give much less color. If too high a concentration of perchloric acid is used, color fading may be produced.

Ferric nitrate is used as a source of ferric ions. Other ferric salts such as perchlorate or sulfate can be used.

Urea "solubilizes" the protein of serum (breaks hydrogen bonds), preventing precipitation of the protein by the strong mineral acid. If too much urea is added, color intensity is diminished. Also at increased concentrations, colorless crystals of unknown composition are likely to appear in the reagent on standing, especially at low temperatures.

Potassium thiocyanate plus mercuric perchlorate reacts to produce an equivalent amount of dissolved mercuric thiocyanate. The solid salt mercuric thiocyanate as purchased is very insoluble and is difficult to put into solution when added as such. During preparation of the color reagent, addition of thiocyanate to the solution containing ferric ions produces a very deep red color, almost black, before addition of mercuric ions. As the addition of mercuric perchlorate (mercuric ions) approaches an equivalent amount, the color decreases rapidly. The endpoint is quite sharp, and should not be passed. Sodium or ammonium thiocyanate may be substituted for the potassium salt.

The dilute solution of mercuric perchlorate (0.02 M) is used for the exact addition of a known excess of mercuric ions.

The solution contains mercuric thiocyanate, free ferric ions, and a small excess of mercuric ions. When chloride ions are added, they first combine with all of the excess mercuric ions, and then with part of the mercuric portion of the slightly ionized mercuric thiocyanate, to form even-less-ionized mercuric chloride. After all of the free mercuric ions have reacted, thiocyanate is liberated stoichiometrically to form ferric
thiocyanate, which can be determined by its light absorption in the neighborhood of its maximum absorbance, 470 m\( \mu \). The color of the ferric thiocyanate is greatly intensified in the strong perchloric acid. Because of this great intensification of color, it is not practical to measure the light absorption that would be produced if all of the approximately 10 \( \mu \text{Eq.} \) of added chloride freed an equivalent amount of thiocyanate to form ferric thiocyanate. Inclusion in the color reagent of a selected amount of free mercuric ions allows binding of an equivalent amount of the added chloride as mercuric chloride before any color is produced. After sufficient chloride is added to react with the excess mercuric ions, color production increases very rapidly, so that a relatively small amount of additional chloride covers the entire practical range of light absorption, with a consequent enhancement of sensitivity to small variations in total chloride content.

Specifically, if 0.1000 ml. of a standard solution of sodium chloride is mixed with 10.00 ml. of color reagent as directed below, no color is obtained unless the chloride concentration of the standard solution is greater than approximately 60 mEq./L. (6 \( \mu \text{Eq.} \)/0.1000 ml.). Thus a curve showing absorbance of the ferric thiocyanate color at 470 m\( \mu \), plotted against chloride concentration in the standard solution, gives a straight line intersecting the \( x \)-axis (chloride concentration) at about 60 mEq./L., as shown by the curve on the right in Fig. 1. The effect of modifying the color reagent by omitting the addition of excess mercuric ions is indicated by the curve on the left of Fig. 1.

**Procedure for Determining the Chloride Content of Serum**

Directions are given for a single analysis, not in duplicate, to be run in 15-mm. diameter cuvets. We have used 125- by 15-mm. "culture tubes" (Corning) selected from large numbers for optical uniformity.

Label 5 clean, dry cuvets: \( B \), \( S_{50} \), \( S_{100} \), \( S_{125} \), and \( Unk \). Into each tube, pipet exactly 10 ml. of color reagent. See that all tubes are maintained at the same temperature (e.g., in a beaker of water at room temperature). Using pipets calibrated to contain 0.1000 ml., add this amount of each of the dilute sodium chloride standards (80, 100, and 120 mEq./L.) to the respective cuvets. In each case the solution is mixed by drawing it back and forth into the pipet 6–8 times. Then stopper the tube with a tight polyethylene stopper and invert several times to mix. Rubber or cork stoppers should not be used.

For the unknown (serum) tube (\( Unk \)), after measuring the serum carefully in the pipet, blow it into the reagent; then mix as described above. Stopper with a polyethylene stopper and invert several times. Again rinse the pipet several times and invert the cuvet to mix. Slow
addition of serum produces a white precipitate or coagulum which can be dissolved only by prolonged mixing.

After the tubes have stood for a few minutes, and are at the same temperature, read in the photometer at a convenient wave length be-

Fig. 1. Curve on right, net absorbances at 470 m\(\mu\) plotted against respective concentrations of chloride in fluid analyzed, using standard color reagent containing 15 mmole of ferric iron per liter, and prescribed 0.30 mmole of free Hg\(^{2+}\) ion per liter added in excess of thiocyanate equivalence. Curve on left, similar results obtained with modified color reagent to which free Hg\(^{2+}\) ions were not added in excess of amount required to combine with all of thiocyanate.

tween 470 and 540 m\(\mu\). Record the total absorbance as \(A_t\). Add 2 small drops (not less than 0.02 ml nor more than 0.04 ml) of 0.2 M \(\text{Hg(ClO}_4\text{)}_2\) solution, and invert the tube, using the plastic stopper. All ferric thiocyanate color fades out in the presence of added mercuric ions. The residual absorbance not due to ferric thiocyanate is now determined, and is recorded as \(A_{tr}\) (\(A\)-thiocyanate faded). Standards usually show negligible absorbance after being faded.

Calculations

Let \(U\) represent milliequivalents of chloride per liter of serum (or other fluid being analyzed); \(A_t\), total absorbance before fading the ferric thiocyanate; and \(A_{tr}\), absorbance remaining after fading the ferric thiocyanate. Calculate net ferric thiocyanate absorbance, \(A\), as the difference between \(A_t\) and \(A_{tr}\):

\[
A = A_t - A_{tr}
\]

Net ferric thiocyanate absorbance for the unknown, \(A_u\), is obtained similarly. Calculate \(A\) for both unknown and standards.

Plotting as ordinates the net ferric thiocyanate absorbances of the standards against corresponding chloride concentrations as abscissas
Fig. 2. Diagrammatic curve similar to that in Fig. 1, illustrating method of calculating results. Mathematical calculation is shown in text, Equation 3.

gives a straight line like that in Fig. 2. Such a "standard curve" can be used to convert graphically values of $A_u$ for various specimens into chloride concentrations. Alternatively, mathematical calculation can be carried out as follows:

Let the factor $F$ represent the reciprocal of the slope of the standard curve as determined by $A_{80}$ and $A_{120}$ (Fig. 2). Then

$$F = \frac{120 - 80}{A_{120} - A_{80}} = \frac{40}{A_{120} - A_{80}}$$

Similarly,

$$F = \frac{U - 100}{A_u - A_{100}}$$

whence

$$U = (A_u - A_{100}) \cdot F + 100. \quad (3)$$

Indicated operations must conform carefully to algebraic signs.

The relationships shown above enable all of the useful range of light absorbance to be employed over the relatively short chloride range, above 60 mEq./L., that is of interest in serum and other biologic fluids of similar chloride content. The choice of the 3 standards at 80, 100, and 120 mEq./L., respectively, covers approximately the range of concentrations of interest in the analysis of blood serum.

**Modifications of the Color Reagent**

The color reagent may be modified for use in various instruments and for the analysis of fluids with contents of chloride lower than that of
serum. In the work here reported, the cuvets used were for the most part of cylindrical test-tube type, although cuvets made of quartz and having parallel sides and a light path of 1.000 cm. through the liquid were also used on occasion. Our test-tube cuvets showed an absorbance corresponding to an integrated length of the light path through the liquid in them of 1.26 cm. Photometers used included the Evelyn type, * Spectronic 20, † the spectrophotometer PMQ II, ‡ and the spectrophotometer Model 15.§

The photometric characteristics of the curves shown herein (like that on the right side of Fig. 1), which do not pass through the origin at zero absorbance, require a slight modification of the concept of molar extinction usually employed (2). The usual concept indicates the absorbance of light of a specified wave length calculated as passing through 1 cm. of a solution of unit molar concentration. In the present paper this concept is modified, so that "molar extinction" implies the change in light absorbance per centimeter of light path that is calculated to result from a 1-molar change (difference) in the concentration of chloride in the contents of the photometer cuvet after enough chloride has been added to react with all of the free mercuric ions in the reagent. An example will make the calculation clear: In securing data for the curve on the right of Fig. 1, 0.1000 ml. of a solution containing 80 mEq./L. of chloride was added to 10 ml. of color reagent to yield 10.10 ml. of colored solution containing 8 μEq. of chloride. In a cuvet with an equivalent light path of 1.26 cm., the absorbance was 0.252 at 470 mμ. Similarly, a standard solution containing 120 mEq./L. yielded 10.10 ml. of colored solution containing 12 μEq. of chloride, with an absorbance of 0.660. Thus, in an effective light path of 1.26 cm., a change in concentration of chloride of 4 μEq./10.10 ml. produced a change in absorbance of 0.660 − 0.252 = 0.408. Then

\[
0.408 \times \frac{1}{(4 \times 10^{-6}/10.10) \times 1000} \times \frac{1}{1.26} = 8.18 \times 10^2 = ε
\]

the molar extinction, according to the modified definition given above.

Circumstances might be encountered in which a lesser molar extinction would be desirable. For instance, if it were desirable to use a cuvet of considerably greater diameter, a reduction in the molar extinction would make it possible still to add 0.1000 ml. of serum to 10.000 ml. of modified color reagent and then to measure the absorbance in the

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*Minneapolis-Honeywell Regulator Co., Denver Div., Denver; modified to take smaller tube.
†Bausch & Lomb, Rochester, N. Y.
‡Carl Zeiss, Inc., New York, N. Y.
larger tube. It is easy to modify the composition of the color reagent to change its molar extinction, merely by changing the content of ferric ions, other constituents remaining the same. The standard color reagent described contains 15 mmole of ferric nitrate per liter, and yields a molar extinction of the approximate magnitude indicated above.

Modified color reagents were prepared containing several respective concentrations of ferric ions. These modified reagents were used to prepare standard curves. Absorbances versus concentrations of chloride in the analyzed solutions are plotted as the family of curves in Fig. 3. It should be noted that the intercept on the x-axis remains essentially unchanged when only the ferric ion content of the reagent is varied. Molar extinctions of the various modified reagents are plotted against their respective iron contents to give the curve shown in Fig. 4. Using the data indicated by this curve, one can make up a reagent with sensitivity suitable for various analytical conditions.

Factors Affecting Results

Effect of Nitrate Ion in the Absence of Urea

If a modified reagent is prepared to contain ferric nitrate without urea, the nitric acid produced from interaction of the nitrate with perchloric acid oxidizes the thiocyanate after it is freed from mercuric ion.

Fig. 3. Family of curves relating chloride concentrations in standards used and respective net absorbances observed at 470 m
dEach straight line was obtained with modified reagent containing, per liter, number of millimoles of iron indicated by figure beside curve, but otherwise having composition of standard color reagent.
The color due to ferric thiocyanate decreases rapidly. Relatively small amounts of urea are sufficient to slow this oxidative action of the nitrate ion greatly, virtually eliminating it. If a modified reagent containing no urea is desired, it is possible to substitute for ferric nitrate another salt, such as ferric ammonium sulfate. Reduction of the urea content increases the molar extinction for a given concentration of ferric ions. The ability to dissolve protein and still yield a clear solution is, however, decreased with decrease in the urea content.

**Presence of Other Substances That Affect Light Absorption**

**Lipemic Serum**

When a lipemic serum is analyzed, the absorbance obtained is, of course, increased above that produced by the ferric thiocyanate alone. When the latter color is then faded by the addition of a slight excess of mercuric ions, as directed, the residual absorption of light by the lipemia is not changed, at least for a while, and can be determined by a second reading in the photometer. The difference between the two absorbances is the net absorbance produced by the ferric thiocyanate alone.

**Hemoglobin**

Serum containing hemoglobin derivatives behaves in a way similar to that described above for lipemic serum. The effectiveness of the compensation for interference due to hemoglobin was demonstrated as follows. A dialyzed solution of oxyhemoglobin was prepared, containing 15 mg./ml. This solution was mixed with an equal quantity of serum.
The mixture was analyzed by adding 0.200 ml. of it to 10 ml. of color reagent. Water (0.100 ml.) was added to the standards to bring them also to the same total volume of 10.20 ml. Measurement of the total absorbance, fading of the thiocyanate with a small excess of mercuric ions, and determination of the residual absorbance was carried out as usual. From the net ferric thiocyanate absorbance, the concentration of chloride in the serum was calculated. Values so obtained on a series of specimens agreed within 1–2 mEq./L. with the values obtained by analyzing the same respective clear, colorless serum specimens in the usual way with no added oxyhemoglobin.

**Bilirubin**

Bilirubin light absorption is not affected by the presence of excess mercuric ions. Hence its presence does not affect the net ferric thiocyanate absorbance, and does not prevent accurate determination of chloride. This fact was confirmed by analyzing tungstic acid filtrates of strongly icteric serums by the modification described below. The pigment is not present in the filtrate, being removed with the proteins.

**Cholesterol**

Besides the possibility that lipemia, hemoglobin, or bile pigment may be present, there is an additional reason for fading the ferric thiocyanate and measuring the residual absorbance of the faded solution when serum is being analyzed. Cholesterol reacts with the color reagent to yield a colored product. After fading, tubes containing serum have a slight yellow color. This residual color is not seen when serum protein-free filtrate, cerebrospinal fluid, or urine is analyzed. Indications are that the yellow color is produced by a reaction between the cholesterol of serum and the ferric ions of the reagent in the strongly acidic solution. If 0.1000 ml. of a serum sample is added to each of several tubes containing color reagent, and successive tubes are allowed to stand for increasing lengths of time before being faded, it is observed that the yellow color obtained on fading increases at a slow rate over several hours, in a nonlinear manner. Excess mercuric ions do not fade this yellow color. Hence by taking the difference between absorbances before and immediately after fading with mercuric perchlorate, one obtains the true net absorbance due to ferric thiocyanate. An accurate analysis for chloride results. Though it is not necessary to do so to produce accurate analyses, the absorbance due to the yellow color can be diminished by making readings at wave lengths greater than 470 m\(\mu\) (maximum absorbance for ferric thiocyanate in the environment used), such as at 500 or at 515 m\(\mu\).
Precipitates and Turbidities

When reagents were prepared with proportions of constituents appreciably different from those stated herein, or when the reagent was stored in the cold, a crystalline precipitate was sometimes observed in it. The composition of the crystals was not determined. In preparation of the reagent, if it is allowed to become too warm, or to stand too long in a partially completed condition, a slight turbidity sometimes develops, which may slowly disappear on continued stirring over a period of half an hour or more. It is better to make the reagent without undue delay, maintaining good stirring during the process, and keeping the temperature of the constituents before and after mixing at approximately 20° until the preparation has been completed.

Especially if old serum is analyzed, a turbidity sometimes develops after the ferric thiocyanate color is faded out. This turbidity is, over a short range, roughly proportional to the excess mercuric perchlorate added. Some of the effect is perhaps related to the freeing of sulfhydryl groups in the protein during aging. These free -SH groups are then bound by the excess mercuric ions. If the amount of excess mercuric salt added does not exceed the prescribed amount, and if the absorbance after fading is determined promptly, this type of slowly-forming turbidity appears not to affect appreciably the accuracy of the chloride determination.

Effect of Larger Proportions of Water

The specific effect of concentrated perchloric acid in intensifying the absorbance due to ferric thiocyanate was shown in two ways.

1. In the first, several modified reagents were prepared having various respective proportions of water substituted for equal volumes of 60% perchloric acid. Chloride standards were added to these reagents in the proportion of 0.1000 ml. of standard per 10.00 ml. of modified color reagent. Absorbances observed with 12 $\mu$Eq. of chloride per 10.10-ml volume and also with 8 $\mu$Eq. per 10.10-ml volume were plotted against various percentages by volume of the perchloric acid in the color reagent replaced with water. The curves so obtained are shown in Fig. 5. The marked decrease in absorbance consequent to decrease in acid content of the reagent is clearly demonstrated.

2. In the second approach to this problem, a constant amount of chloride (10 $\mu$Eq.), contained in various volumes of water, was added to respective 10.00-ml amounts of color reagent made as directed in Procedure for Color Reagent. These additions yielded corresponding various volumes of solutions whose colors were produced in each case by 10 $\mu$Eq. of chloride in the total volume. In Fig. 6 (lower curve) is
shown the curve obtained by plotting absorbances produced by this constant amount of chloride against volumes of the aqueous chloride solutions added to the 10-ml volume of color reagent. The total volume was the volume of aqueous solution added plus 10 ml. The upper curve of

![Graph showing absorbances produced by chloride in aqueous solutions of perchloric acid in color reagent.](image)

**Fig. 5.** Effect of perchloric acid in intensifying color of ferric thiocyanate when reagent was replaced by water. To 10-ml volumes of various reagents modified by replacement of part of perchloric acid with water were added respectively 0.1 ml of either 0.08 M NaCl or 0.12 M NaCl solutions.

Fig. 6 shows absorbances to be expected if dilution up to the larger volume were the only cause of change. The differences between the ordinates of these 2 curves show the decrease in absorbance caused by dilution of the perchloric acid and the other constituents (all in the same proportion) by the added water.

**Analysis of Fluids Other Than Serum**

*Protein-free Filtrates of Serum or Plasma*

It may be desirable to analyze protein-free filtrates of serum or plasma, such as a 1:10 tungstate filtrate, for instance. Such filtrates have to be used in larger volume (1.00 ml. of a 1:10 filtrate), and the increased volume of water added will affect the chromogenicity, as discussed above and as illustrated in Fig. 6. Accurate results can be obtained, provided the chloride standards are diluted to the same extent as are the filtrates and are added in the same volume of water as that in the filtrates added. Since cholesterol, hemoglobin, bile pigments, and lipids are removed with the proteins when the filtrates are prepared,
these substances do not contribute to the total absorbance, nor does any yellow color remain when the ferric thiocyanate color is faded by mercuric ions. In fact, in analyzing protein-free filtrates, it is not necessary to fade either the unknowns or the correspondingly diluted standards in order to obtain satisfactory results, the total absorbances being taken as equal to the net ferric thiocyanate absorbances. Results agree with those obtained by the prescribed procedure using whole serum.

**Cerebrospinal Fluid**

Cerebrospinal fluid is analyzed in the same way as is whole serum, except that fading is unnecessary if the fluid is clear and colorless. Because of higher concentrations of chloride in cerebrospinal fluid than in serum, it may be advantageous to use a slightly modified formula instead of that shown in Equation 3, as follows:

\[ U = (A_u - A_{120}) \cdot F + 120. \]  

(4)

**Urine**

As shown in Fig. 1, the range of concentrations of chloride which can be determined within usable limits of absorbance is rather small when the reagent has the composition first herein described. Fig. 1 also indicates (curve on the left) that modification of the composition by omitting the addition of excess mercuric ions to the reagent allows the analysis of fluids containing about 10–50 mEq. of chloride per liter, provided
corresponding standards are employed. With such a modification, dilute urine can be analyzed, and concentrated urines can be diluted by trial and error to fall within the range of 10 to 50 mEq./L.

It is possible, moreover, to modify the reagent in another way, increasing considerably the range of concentrations over which it can be used. For this purpose a reagent was prepared that differed from the standard color reagent in two respects: (1) it contained only 5 mmoles of ferric ion per liter; and (2) it contained no added excess of mercuric ions. These modifications produce a reagent suitable for the analysis of fluids having chloride contents from 10 to as high as 200 mEq./L. (using 0.1000 ml. of fluid plus 10 ml. of reagent). Corresponding absorbances were 0.046 to 0.979 for test tube cuvets 15 mm. in diameter (with equivalent liquid light path of 1.26 cm.), at 470 mJ. The curve relating absorbances and concentrations is not linear with this modified reagent. Translation of absorbances observed must therefore be made by reference to a curve prepared by using suitable standards. Results obtained are less accurate than are those obtained with the standard color reagent.

The Effects of Other Halogens

It is emphasized that the equilibriums shown in Equations 1 and 2 are dynamic ones, and represent diminutions in ionizations, but not completion of reactions. Bromide ion appears to combine more strongly with mercuric ion than does chloride. Ten microequivalents of bromide (in 0.1 ml.) added to 10 ml. of color reagent produces a greater absorbance from the thiocyanate set free from its combination with mercury and reacting with ferric iron than does an equivalent amount of chloride. Using the "standard" color reagent containing 15 mmoles of ferric iron per liter, the molar extinction produced by bromide is about 1.6 times as great as is that produced by chloride. Therefore, when bromide is present in serum, the determination by this method of the apparent number of milliequivalents of chloride per liter will give results in positive error for chloride by somewhat more than the concentration of bromide present. In other words, the analytical result will be slightly higher than the sum of the numbers of milliequivalents of both ions present. If one-third of the chloride ions in serum are replaced by bromide ions, as may occur in bromide intoxication, the bromide ions present combine with the excess, or free, mercuric ions in the reagent to a greater extent than do chloride ions. Likewise, some of the chloride added to the reagent as mercuric chloride is displaced by bromide. These shifts in equilibrium result in the number of "free" bromide ions now being less than one-third of the total halogen ions. Thus the analytical error that might be
predicted solely from a consideration of the relative molar extinctions produced by bromide and by chloride is diminished. On the basis of dilution experiments, it appears that the over-all error will be such that a serum containing, per liter, 65 mEq./L. of chloride and 35 mEq./L. of bromide would be found by this method of analysis to have, per liter, about 108 or 110 mEq./L. of “chloride” (halogen).

Fluoride in large quantities, such as may be present when fluoride is used in an anticoagulant mixture, has a marked deleterious effect on the method, apparently by binding the ferric iron. In concentrations insignificant with respect to the amount of iron in the reagent, fluoride has no effect.

Effects of Temperature

The absorbance of a solution prepared by adding 0.100 ml. of 0.1 M NaCl to 10 ml. of color reagent varies about 7–8% per 10-degree change in room or bath temperature. This characteristic makes it necessary to keep the standard tubes and the unknowns at the same temperature during the determination. It is usually satisfactory to immerse the photometer tubes in a beaker of water at room temperature.

The color reagent is stable for several months if kept at a temperature in the neighborhood of 20°. On prolonged storage at 25°, and considerably more rapidly at 35–40°, the molar extinction produced by a given quantity of chloride diminishes considerably, probably due to changes in the thiocyanate.

Accuracy

The accuracy (reproducibility) of the method depends largely upon technical points and upon observance of directions given. In the comparative analyses listed herein, pipets as purchased were used. Determination of their accuracy of calibration showed deviations of up to 0.8% in the volumes of 0.1-ml. pipets used. Naturally, analytical results would vary by an even greater amount when a nominal 0.1000-ml. pipet might contain 0.0992 or 0.1006 ml. of serum. Furthermore, unless the analyst has had experience in measuring and handling this size of sample, manipulative errors may occur. Photometric errors, on the other hand, are less than those encountered in most methods, because of the magnitude of change of the absorbance with relatively small change in concentration of chloride.

Comparison with Other Methods

The primary reference method used was the titrimetric silver iodate method of Sendroy (5). A series of specimens of serum were obtained
Table 1. Results Obtained in Human Serum by the Titrimetric Silver Iodate Method and Mercuric Thiocyanate Method

<table>
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<tr>
<th>Cl⁻ (mEq./L. of serum)</th>
<th>Ag⁺⁺⁺ method</th>
<th>Hg(SCN)²⁻ method</th>
<th>Error*</th>
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<td>98.4</td>
<td>96.7</td>
<td>+ 0.3</td>
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<td>99.9</td>
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<td>105.0</td>
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<td>95.8</td>
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<td></td>
</tr>
<tr>
<td>106.2</td>
<td>107.9</td>
<td>+ 1.7</td>
<td></td>
</tr>
<tr>
<td>106.1</td>
<td>107.4</td>
<td>+ 1.3</td>
<td></td>
</tr>
<tr>
<td>104.0</td>
<td>105.5</td>
<td>+ 1.5</td>
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<tr>
<td></td>
<td>104.3</td>
<td>+ 0.3</td>
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</tr>
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</table>

*Assuming Ag binding gives correct values.

from hospital patients. These were analyzed in duplicate by the Sendroy method. The average of these duplicates is presented in Table 1. Analyses were then made by the mercuric thiocyanate method described herein, each photometer tube being allowed to stand for 30 min. before being read in the photometer, then faded with mercuric perchlorate, and read again. Results are given in Table 1, together with the deviation of the mercuric thiocyanate value from that obtained by the silver iodate method. The series presents consecutive analyses, with no omissions.

The mercuric thiocyanate method of Schoenfeld and Lewellen (4) employs nitric acid instead of perchloric acid, and develops a considerably lower molar extinction with chloride. Comparative results for chloride in serum are given in Table 2 for their method and for the one presently described. Again a consecutive series is given.

Precautions To Be Observed

Anhydrous perchloric acid is unstable and may explode spontaneously as a result of the accumulation of chloric acid, especially in the presence of organic matter. When the acid is diluted with water, as is the 60% acid employed herein, it ceases to have any explosive action, and at room temperatures it does not even act as an oxidizing agent. The color reagent described is about 5.5 N in perchloric acid. Small amounts of the
Table 2. Results Obtained with the Use of Nitric Acid and Perchloric Acid

<table>
<thead>
<tr>
<th>Schloenfeld and Lewellen method</th>
<th>Hamilton method</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>104.9</td>
<td>105.5</td>
<td>-0.6</td>
</tr>
<tr>
<td>106.1</td>
<td>106.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>102.5</td>
<td>102.7</td>
<td>-0.2</td>
</tr>
<tr>
<td>101.1</td>
<td>100.8</td>
<td>+0.3</td>
</tr>
<tr>
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<td>88.3</td>
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</tr>
<tr>
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<td>+0.5</td>
</tr>
<tr>
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<td>103.4</td>
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<tr>
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</tr>
<tr>
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<td>108.7</td>
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</tr>
<tr>
<td>107.1</td>
<td>107.0</td>
<td>+0.1</td>
</tr>
</tbody>
</table>

reagent have been evaporated and ignited in an open crucible without accelerated burning or explosion. When the reagent is spilled on cloth or on paper and is allowed to remain, it makes a hole, as does sulfuric acid. If paper contaminated with the reagent is burned, the portion with the acid solution on it flares up. Hence paper or cloth on which the reagent has spilled should be washed in running water or treated with sodium bicarbonate, and not merely discarded in the contaminated condition.

Because of the strong acidity, contact with eyes, mucosa, or skin should be avoided, and if some of the solution gets on the hands or on clothing, it should immediately be washed off with large amounts of water, or rinsed with a solution of sodium bicarbonate.

References