United States Patent Office 3,185,549
Patented May 25, 1965

3,185,549

COLORIMETRIC DETERMINATION OF CHLORIDES IN SERUM

Robert H. Hamilton, Philadelphia, Pa., assignor to Hartman-Leddon Company, Inc., Philadelphia, Pa., a corporation of Delaware

No Drawing. Filed Aug. 27, 1962, Ser. No. 219,732
12 Claims. (Cl. 23—230)

This invention relates to a reagent and method for determining the presence of small amounts of chloride in serum, the reagent and its method being suitable for making either visual or spectrophotometric comparisons in determining minute amounts of chloride.

A principal object of the present invention is to provide a method and a reagent useful for making accurate colorimetric determinations of chloride in blood serum and without producing precipitates which would interfere with or prevent the obtaining of meaningful test results. In this connection, it is a specific object to provide a method based on the displacement of thiocyanate from mercuric thiocyanate by chloride ions and the subsequent reaction of the liberated thiocyanate with ferric ions to form the colored ferric thiocyanate complex, the color produced by such a reaction being intensified by the presence of perchloric acid without the attending precipitation of proteins by that acid. Another object is to provide a direct photometric method and reagent for determining the presence of chloride in serum in which the color development is substantially linear over the range to be tested. Other objects will appear as the specification proceeds.

An important aspect of the present invention lies in the discovery that the presence of chloride in serum may be determined colorimetrically, without the formation of a protein precipitate, if a measured quantity of urea is included in the testing solution. Further, it has been found that the presence of urea permits the use of strong acids which would otherwise cause a precipitation of protein, and in particular, permits the use of concentrated perchloric acid. While other strong mineral acids may be used, such as sulfuric or nitric acids, perchloric acid is especially desirable because it greatly intensifies the color development.

It has for some time been known that the presence of chlorides may be detected colorimetrically by treating a sample with a test solution of perchloric acid containing mercuric thiocyanate and ferric ions. The fundamental reactions that take place are as follows:

(1) $2Cl^- + Hg(SCN)_2 \rightarrow HgCl_2 + 2SCN^-$

(2) $SCN^- + Fe^{+++} \rightarrow FeSCN^{++}$ (red)

These reactions require the presence of a strong mineral acid such as perchloric acid. While various concentrations may be used, it has been found that the color of the ferric thiocyanate is greatly intensified if the final perchloric acid concentration is within the range of 35 to 45 percent, the optimum concentration being approximately 41 percent. The results at acid concentrations below this range tend to be erratic and at higher concentrations the color developed with serum tends to fade. Generally, perchloric acid concentrations in the lower range can be tolerated if spectrophotometric measurements are to be made rather than visual comparisons.

Ferric perchlorate is preferred as a source of ferric ions. Other ferric salts can be used, however, as long as they do not introduce extraneous anions which will behave adversely in the strong acid medium.

Since mercuric thiocyanate is not readily soluble as such, it has been found desirable to add measured amounts of potassium thiocyanate and mercuric perchlorate to the acid solution, the two ingredients thereby producing a known quantity of dissolved mercuric thiocyanate. Preferably, a slight excess of mercuric perchlorate is provided since the presence of additional mercuric ions serves to limit color development by requiring chloride ions in excess of the mercuric ions before a color change occurs. When a sample containing a sufficient quantity of chloride ions is reacted with the test solution so that the red color of ferric thiocyanate appears, the steep curve of color development with increasing amounts of chloride permits an accurate chloride determination without reaching optical densities beyond the range of ordinary photometers.

In addition to the above ingredients, the test solution also contains urea and a small amount of mercuric chloride. Generally, the amount of urea, when measured volumetrically as a 50 percent weight to weight aqueous solution, should be at least equal to 25 times the volume of serum of the test sample. Preferably, the volume of the added urea solution should range between 30 to 45 times the serum volume. Since normal testing procedure involves 10 milliliters of testing solution and 0.1 milliliter of serum, it has been found that a suitable testing solution may be prepared by using approximately 170 to 250 grams of undiluted urea for each liter of final solution. An optimum amount of urea is found to be approximately 200 grams per liter of final solution or, when the urea is previously diluted with an equal part by weight of water, a volume of approximately 350 milliliters for each liter of final solution.

The urea solubilizes the protein of the serum and prevents it from being precipitated by the perchloric acid of the testing solution. Thus, if insufficient amounts of urea are present, a protein precipitate or turbidity will be formed and will interfere with or prevent the making of meaningful colorimetric determinations. On the other hand, if excess amounts of urea are present, color intensity is diminished and crystals of unknown composition, possibly urea perchlorate, tend to appear in the testing solution on standing.

The excess mercuric ions added as mercuric perchlorate "subtract" or bind a fixed amount of the chloride to be determined. Removal of this fixed amount of chloride leaves a shorter range of chloride concentrations to react with the mercuric thiocyanate, and thus permits use of the greater color intensity, with a greater change of absorbance per unit change of chloride concentration. Photometric accuracy is thereby enhanced. The microequivalents of excess mercuric perchlorate added should be somewhat less than the minimum number of microequivalents of chloride expected in the samples to be analyzed.

Mercuric chloride in the testing solution improves linearity. The amount of mercuric chloride in the test solution is small and preferably ranges between 0.1 to 0.2 grams for each liter of the solution. Particularly effective results have been found where approximately 0.16 gram of mercuric chloride is used in preparing the final testing solution. This is accomplished by adding 6.0 milliliters of 0.1 molar mercuric chloride to the other reagents in 1 liter of the testing solution. Besides improving the linearity, the amount of mercuric chloride can be used to adjust the absorbance range produced after color development, in order that a thickness of solution optimum for a specific photometer can be used.

In performing a test, an accurately measured quantity of serum, usually 0.1000 milliliter, is mixed in a photometer tube with exactly 10 milliliters of the reagent solution. Identical quantities of sodium chloride standards (80, 100, 120 meq. per liter) are added and mixed with the reagent solution in three photometer tubes, each tube containing exactly 10 milliliters of the color solution. The tubes are stoppered and, after 20 to 30 minutes, are read in a photometer at a convenient wave length between 430 to 550 millimicrons, such as 515 millimicrons. After the absorbance of the ferric thiocyanate

solution has been determined, color is faded out by the addition to each tube of measured quantities of excess mercuric ions which combine with the thiocyanate of the ferric thiocyanate. Second readings in the photometer then give a "blank" correction due to chromogens not de-colorized by mercuric ions. Hemoglobin derivatives in moderate amounts thus do not interfere with the photometric determination, nor does lipemia.

The method and testing solution or reagent of the present invention are further disclosed in the following illustrative example:

Example

One liter of a color reagent or testing solution embodying the present invention may be prepared from the following ingredients:

	Ml.
Urea, 50% (w./w.)	350
$HClO_4$, 60%	600
$Fe(ClO_4)_3$, approx. 0.5 g./ml.	10.0
KSCN, 0.4 M	12.5
$Hg(ClO_4)_2$, 0.2 M; add to an exact colorless end-point; requires about	12.5
$Hg(ClO_4)_2$, 0.02 M	15.0
$HgCl_2$, 0.1 M	6.0

To prepare this solution, 500 g. of urea are dissolved in 500 g. (501.5 ml.) of water, with warming to 35–40°. Total volume is about 875 ml. If this solution looks dirty, it can be clarified by mixing it with 1 ml. of 10% $ZnSO_4 \cdot 7H_2O$, followed by 1 ml. of approximately 0.5 N NaOH. A flocculation which appears may then be filtered out through a coarse-porosity sintered glass filter with washed asbestos fibers (analytical grade). The resulting solution is clear and colorless.

The ferric perchlorate is prepared by dissolving in water 25 g. of a commercial ferric perchlorate, non-yellow, excess perchlorate acid preparation having a salt content of about 50% by weight, and thereafter adjusting the volume to 25 ml.

The 0.2 M mercuric perchlorate is prepared by adding 10 ml. of 60% perchlorate acid to 4.33 g. of pure mercuric oxide (red), and then warming to dissolve. Thereafter, the solution is cooled and diluted to 100 ml. with water. Further dilution of a portion of this solution produces the 0.02 M mercuric perchlorate reagent.

The potassium thiocyanate constituent is prepared by dissolving 3.89 g. of pure potassium thiocyanate in water and then diluting to 100 ml., and the mercuric chloride is similarly prepared by dissolving 2.72 g. of the salt in water and diluting to 100 ml.

After the initial photometric determinations are taken as described above, two drops (about 0.02 to 0.04 ml.) of 0.2 M $Hg(ClO_4)_2$ solution is added to each photometer tube and mixed with the solution contained therein. All ferric thiocyanate color fades out in the presence of the added mercuric ions. The optical density is again determined and recorded as the final reading to give the "blank" correction resulting from chromogens not decolorized by mercuric ions.

While in the foregoing I have disclosed an embodiment of the present invention in considerable detail for purposes of illustration, it will be understood by those skilled in the art that many of these details may be varied without departing from the spirit and scope of the invention.

I claim:

1. In the colorimetric determination of chloride in serum, the steps of reacting chloride in serum with mercuric thiocyanate in a perchloric acid solution containing ferric ions, and simultaneously solubilizing the proteins of said serum in said solution with urea to prevent impairment of colorimetric determinations by reason of protein precipitates without at the same time reducing color intensity.

2. In the colorimetric determination of chloride in serum, the steps of reacting the chloride of serum with mercuric thiocyanate in a perchloric acid solution containing ferric ions, and simultaneously solubilizing the proteins of said serum in said solution with a volume of 50 percent by weight aqueous urea at least equal to 25 times the volume of said serum.

3. The method of claim 2 in which 30 to 45 parts by volume of 50 percent by weight aqueous urea are provided for each part by volume of serum.

4. The method of claim 2 in which said solution also contains a minor proportion of mercuric chloride.

5. In the colorimetric determination of chloride in serum, the steps of contacting serum with mercuric thiocyanate in a strong mineral acid solution containing ferric ions, and simultaneously solubilizing the proteins of said serum in said solution with urea.

6. The method of claim 5 in which the volume of 50 percent by weight of aqueous urea in said solution is at least equal to 25 times the volume of serum.

7. The method of claim 5 in which 30 to 45 parts by volume of 50 percent by weight aqueous urea are provided for each part by volume of serum.

8. In the colorimetric determination of chloride in a test solution, the step of reacting said chloride with mercuric thiocyanate in a strong perchloric acid solution containing ferric ions, the concentration of said perchloric acid in the final solution being within the range of 35 to 45 percent for intensifying the color of the ferric thiocyanate reaction product.

9. A color reagent for the colorimetric determination of chloride in serum, comprising a strong mineral acid solution of mercuric thiocyanate and a ferric salt, said solution also containing 170 to 250 grams of urea dissolved in each liter of said solution.

10. The reagent of claim 9 in which approximately 200 grams of urea are dissolved in each liter of said solution.

11. A color reagent for the colorimetric determination of chloride in serum, comprising a perchloric acid solution of mercuric thiocyanate and a ferric salt, said solution also containing 170 to 250 grams of urea dissolved in each liter of said solution.

12. The reagent of claim 11 in which approximately 200 grams of urea are dissolved in each liter of said solution.

References Cited by the Examiner

UNITED STATES PATENTS

3,055,742 9/62 Hamilton ..... 23—230

OTHER REFERENCES

Wardle: "Increasing Use of Urea in Textile Manufacturing," Textile Colorist, 62, Sept. 15, 1940, pages 629–630.

"Practical Physiological Chemistry," Hawk et al., 12th ed., 1947, pages 573–577 relied on.

MORRIS O. WOLK, *Primary Examiner.*

JAMES H. TAYMAN, JR., *Examiner.*