A NEW COLORIMETRIC PROCEDURE FOR THE DETERMINATION OF SERUM POTASSIUM WITH SILVER COBALTINITRITE REAGENT

SUSUMU SHIBATA AND WATARU MIZUTA

Department of Clinical Pathology, Yamaguchi Medical School, Ube

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There seems to be not much to choose between the cobaltinitrite and the silver cobaltinitrite procedures which are currently employed for the determination of serum potassium, since they exhibit merits as well as demerit equaling each other when they are used for routine purposes[1]. The former procedure[2)3)] is advantageous because of the availability of the photoelectric procedure, in which potassium is precipitated as potassium sodium cobaltinitrite, the precipitate is washed, dissolved in hot water, and an excess of choline chloride and sodium ferrocyanide is added to produce the extremely stable emerald green color of cobaltous choline ferrocyanide. However, its accuracy is regretfully liable to the error which is caused by the loss of precipitate on washing, for potassium sodium cobaltinitrite is slightly soluble in wash solution (water). In the latter method[1)4)] potassium and silver cobaltinitrite produce the precipitatation of silver potassium cobaltinitrite which is so adequately coarse-particled and so much insoluble in water that complete purification is enabled by repeated centrifugation and washing. Unfortunately this excellence is handicapped by the defect that the precipitate is not easily dissolved in hot water. Acid is used to dissolve the sediment of silver potassium cobaltinitrite, and the resultant solution is titrated for nitrite radical with permanganate. When colorimetric procedure is desired, it is diazotized to yield red or yellow color, but the coloration is not suitable for colorimetry because of either the faintness or the unstability of its coloration.

For the past few years coupling of silver cobaltinitrite procedure with the stable coloration of cobaltous choline ferrocyanide which assured photoelectric colorimetry has been sought in our laboratory, and this was finally attained by dissolving the precipitate of silver potassium cobaltinitrite in hot aqueous solution of sodium thiosulfuric acid. Our procedure is convenient for routine work, since precipitation of silver potassium cobaltinitrite by means of a simple stirrer which is specially constructed by hand is also in co-operation with the photoelectric colorimetry which has been mentioned.

Instruments

1) Fifteen ml. centrifuge tubes of pyrex glass which are graduated to 0.2 ml.

2) Stirrer: 1.5 volt magent motor whose rotating axis is equipped with a slender glass rod (2mm. in diameter) by means of a rubber tube. This is illustrated in Figure 1. When the glass rod is immersed in a fluid contained in a centrifuge tube, and the motor is brought into action with a dry cell, the solution is stirred with constant rapidity and completeness, because the glass rod makes a conical rotation with the rubber tube as its vertex or the supporting point of its motion.

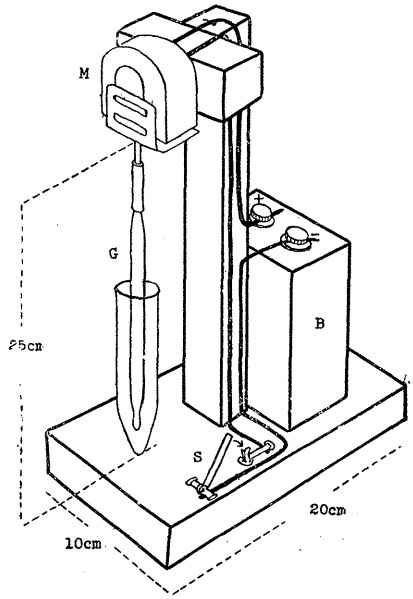

Fig. 1 A stirrer for precipitate formation built with a miniature electric motor.
B: dry cell, G: glass rod, M: miniature motor, S: switch

3) Aspirator: A glass tube with its tip stretched into a J-formed tubule is connected with a water stream pump.

Reagent

1) Standard solution of potassium (1mg. K/ml.): Dissolve in distilled water 2.228 g. of the pure crystals of potassium sulfate (K_2SO_4) which has been dried at 100 C for an hour and allowed to cool in a desiccator and make to volume of 1000 ml. Prepare the solutions of 15, 20 and 25 mg. K/dl. every time on use by dlstilled water.

2) 1.5 g./dl. aqueous solution of sodium tungstate: Dissolve 1.5 g. of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) in distilled water and make to 100 ml.

3) 2.5 g./dl. aqueous solution of copper sulfate: Dissolve 2.5 g. of copper sulfate crystals ($CuSO_4 \cdot 5H_2O$) in distilled water and make to volume of 100 ml.

4) 2.5 g./dl. aqueous solution of silver nitrate: Dissolve 2.5 g. of silver nitrate crystals ($AgNO_3$) in distilled water and make to 100 ml.

5) Silver cobaltinitrite reagent[2)4)]: Mix the whole volume of solution A with 210 ml. of solution B (both solutions are to be described below) and aerate until the mixture no longer smells fishy (complete removal of NO_2 gas).

Filter with a filter paper Tôyôroshi No. 2 before use to obtain clear filtrate.

Agitate vigorously 20 ml. of the filtrate with 1 ml. of solution C (to be described below)

and filter again with a filter paper Tôyôroshi No. 7. The resultant filtrate is the silver cobaltinitrite reagent.

Solution A: Dissolve 25 g. of pure cobaltous nitrate ($Co(NO_3)_2 \cdot 6H_2O$) in 50 ml. of distilled water, add 12.5 ml. of glacial acetic acid (CH_3COOH), and mix completely.

Solution B: Dissolve 120 g. of pure sodium nitrite ($NaNO_2$) in 180 ml. of distilled water.

Solution C: Dissolve 20 g. of pure silver nitrate ($AgNO_3$) in distilled water, make to volume of 50 ml., and preserve in a brown bottle.

6) 1 g./dl. aqueous solution of sodium thiosulfuric acid: Dissolve 5 g. of pure sodium thiosulfuric acid ($Na_2S_2O_3 \cdot 5H_2O$) in distilled water, and make to volume of 500 ml.

7) 1 g./dl. aqueous solution of choline chloride: dissolve 1.0 g. of pure choline chloride ($(CH_3)N\ ClCH_2CH_2OH$) in distilled water and make to 100 ml.

8) 2 g./dl. aqueous solution of potassium ferrocyanide: Dissolve 2 g. of potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) in distilled water, make to volume of 100 ml., and preserve in a brown bottle.

Procedure

(1) Removal of protein: Into a test tube pipette 1.0 ml. of nonhemolyzed blood serum, 4.0 ml. of distilled water and 2.0 ml. of sodium tungstate solution, mix, add 2.0 ml. of copper sulfate solution, agitate thoroughly, add 1.0 ml. of silver nitrate solution, and mix vigorously. Allow the mixture to stand for fifteen to twenty minutes, and filter with a filter paper Tôyôroshi No. 7 to obtain clear deproteinized filtrate which is the ten-fold dilution of the original serum.

(2) Precipitation of silver potassium cobaltinitrite ($K_2AgCo(NO_2)_6nH_2O$): Into centrifuge tubes A, B, C and D transfer the deproteitinized filtrate and the standard solution (15, 20 and 25 mg./dl. K) as listed below.

A. 15 mg. K/dl. solution 0.5 ml. + distilled water 4.5 ml.
B. 20 mg. K/dl. solution 0.5 ml. + distilled water 4.5 ml.
C. 25 mg. K/dl. solution 0.5 ml. + distilled water 4.5 ml.
D. Deproteinized filtrate 5.0 ml.

Incubate the tubes at 20 to 30 C for five minutes, add slowly to each two ml. of the silver cobaltinitrite reagent under constant stirring by means of a stirrer, and continue to agitate for about a minute after the addition is finished in order to complete the precipitation of silver potassium cobaltinitrite. Remove the tubes from the stirrer with caution lest any noticeable amount of precipitate is lost with its glass rod, and allow them to stand at 10–20 C for two hours.

(3) Washing: a) Centrifuge the tubes at 3000 r. p. m. for fifteen minutes, discard the supernatant layer nearly to the mark of 0.2 ml. with an aspirator. b) Slowly pour down about 5 ml. of distilled water on the inner walls of the tubes which are held inclined, and gently stir with special circumspection so that the precipitate which is collected at the bottom of the tubes may not be whirled up. c) Repeat the steps a) b) and a) once more.

(4) Dissolution of precipitate and coloration: Add 5.0 ml. of sodium thiosulfate solution to each of the tubes, stir up the precipitate with a slender glass rod, immerse the tubes in a boiling water bath until the precipitate is dissolved completely, allow to cool at room temperature, add 1.0 ml. of choline chloride solution, mix, add 1.0 ml. of ferrocyanide solution, and mix thoroughly. Subject similarly a volume of 0.2 ml. of distilled water to the procedure of dissolution and coloration. This constitutes the blank solution for colorimetry.

(5) Colorimetry: Measure the absorbance at 610 mμ (or 430 mμ) in a photoelectric colorimeter set to zero with the blank solution. Construct a calibration curve, assuming A, B and C to be 15, 20 and 25 mg. K/dl. in concentration, respectively. Read the concentration for the absorbance of D from this curve. Then the concentration represents the serum potassium desired (in mg./dl.).

The accuracy of our procedure was examined as follows.

1) The light absorption and the obedience to Beer's law of the cobaltous choline ferrocyanide solution (Experiment 1): Standard solutions of 10, 20 and 30 mg.K/dl. were treated strictly by the whole course of the steps of our procedure, and the absorbance of the resultant colored solutions was measured over the entire range of visual spectrum (430-690 mμ) in Erma's photoelectric spectrophotometer (square cuvette of 1.0 cm. optical path).

2) Duplicate determination and recovery test (Experiment 2): Fifteen blood serums were determined in duplicate for potassium concentration by our procedure in order to ascertain the range of divergence.

Volumes of 0.5 ml. of 6 mg.K/dl. potassium sulfate solution (K≡30γ) and similarly 0.5 ml. portions of the 5 mg.K/dl. solution (K≡25γ) were added to ten aliquots of 5.0 ml. of the deproteinized filtrates (ten-fold dilution of the original serums; K content is about 100γ), five specimens for each group, to examine the amout of recovery.

Results

Figures 2 and 3 represent the data of the experiment 1. From these it is readily recognized that the cobaltous choline ferrocyanide solution has two maximums of light absorption, at 430 mμ and 630 mμ, respectively, and that Beer's law is strictly followed at the latter wave length, while at the former it is also obeyed in approximation.

The divergence in duplicate determination (Experiment 2) was 0.2 mg./dl. (Standard deviation 0.2), and the recovery amounted to 103.2% (S.D. 4.5) and 99.2% (S.D. 10.3) for the addition of 30γ and 25γ of potassium, respectively.

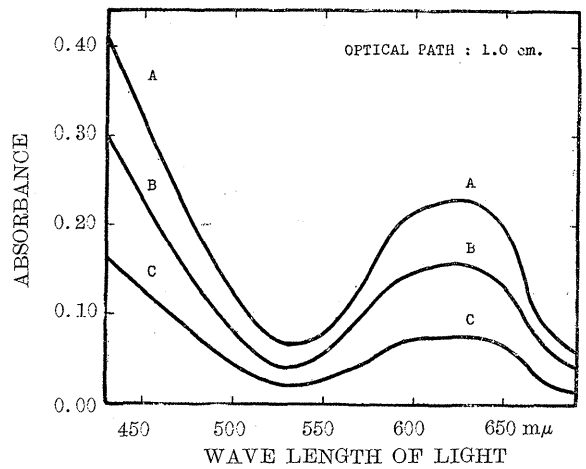

Fig. 2. Light absorption of cobaltous choline ferrocyanide solution.

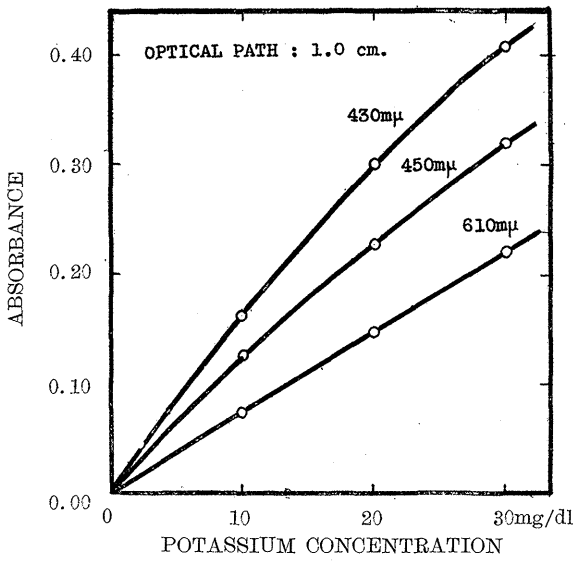

Fig. 3. Obedience to Beer's law at 610 mμ.

The normal range of serum potassium was 17.3 ± 0.8 mg./dl. (confidence interval with confidence coefficient $(1-\alpha)=95\%$).

Summary and Conclusion

A new procedure for the determination of serum potassium was presented. Potassium was precipitated by the addition of silver cobaltinitrite solution to serum, the precipitate thus formed was purified by centrifugation and washing, and dissolved in sodium thiosulfate solution. The resultant solution was treated by choline chloride and potassium ferrocyanide to produce a emerald green color which was suitable for photoelectric colorimetry. This procedure was recommended for the routine laboratory work, because it was simple as well as reliable.

References

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