United States Patent Office 3,457,045
Patented July 22, 1969

3,457,045

QUANTITATIVE DETERMINATION OF SERUM CALCIUM

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No Drawing. Filed Apr. 25, 1966, Ser. No. 544,837
Int. Cl. G01n 33/16, 31/02
U.S. Cl. 23—230 6 Claims

ABSTRACT OF THE DISCLOSURE

Composition and process for quantitatively determining the amount of calcium ion in blood serum. A measured quantity of blood serum is treated with an aqueous solution of measured quantities of sodium rhodizonate and sulfosalicylic acid; proteins and interfering chromogens from the blood serum are precipitated out by the sulfosalicylic acid. The clear solution remaining after the removal of the precipitate contains a colored calcium rhodizonate formed by the reaction of the sodium rhodizonate with substantially all of the calcium ions present in the blood serum. The reaction is quantitative and the intensity of the color, as measured at a wave length of 550 mμ, is compared against known standard solutions of calcium rhodizonate. The addition of the sulfosalicylic acid and sodium rhodizonate solution to the blood serum being tested can be performed consecutively, but for convenience, simultaneous addition of both reagents is preferred.

The present invention relates to a composition and process for determining quantitatively the amount of calcium ion in blood serum, and more particularly relates to the use of sodium rhodizonate as a diagnostic agent for the quantitative determination of serum calcium.

Sodium rhodizonate is known to be useful as a qualitative agent for the determination of the presence of alkaline earth metals such as barium, strontium and calcium in unknown chemical samples.

It has been discovered that sodium rhodizonate (i.e., the disodium salt of rhodizonic acid) reacts specifically and quantitatively with serum calcium to produce a highly colored compound, the intensity of color of which depends on the concentration of calcimum ion present in the blood serum being tested, i.e., on the amount of calcium rhodizonate formed. The resulting colored solution can then be compared against known standard solutions of calcium rhodizonate at a wave length of 550 mμ on a spectrophotometer or a colorimeter to determine the quantity of calcium ions present in the blood serum sample.

The instant procedure is specific for calcium in blood serum since there are no significant quantities of any other alkaline earth metal ions such as barium or strontium ions in blood serum.

The determination of the amount of calcium ion in blood serum is, of course, an important diagnostic tool in the determination of certain physiological disorders in the animal body such as those causing an electrolyte imbalance, impairment of calcium utilization, etc.

The process of the invention is carried out by reacting

a given quantity of blood serum with from about 0.0204 to about 0.0276, preferably about 0.0216 to about 0.0264 part of sodium rhodizonate and from about 2.55 to about 3.45, preferably 2.70 to about 3.30 parts of sulfosalicylic acid, preferably as the dihydrate, made up into an aqueous solution with distilled or deionized water. The above parts are parts by weight per part by weight of blood serum. The above aqueous solution contains from about 0.00136 to about 0.00184 part by weight, preferably about 0.00144 to about 0.00176 part by weight of sodium rhodizonate and from about 0.17 to about 0.23 part by weight, preferably about 0.18 to about 0.22 part by weight of sulfosalicylic acid, per part by weight of water. The sulfosalicylic acid dihydrate is employed to precipitate proteins and interfering chromogens from the blood serum. For convenience, the sulfosayicylic acid dihydrate is employed simultaneously with the sodium rhodizonate in the same solution, although, if desired, all of the sulfosalicylic acid dihydrate can be dissolved in about half of the above quantity of water and mixed with the blood serum, followed by the addition of all of the sodium rhodizonate dissolved in the other half of the water.

The above solution of sodium rhodizonate and sulfosalicylic acid, or the separate solutions of each, are mixed with the sample of blood serum and the precipitated protein and chromogens are removed, e.g., by centrifugation or filtration. The resulting clear solution is then added to from about 3 to about 13 parts, preferably about 7 to about 9 parts by volume of a solution of from about 40% to about 75%, preferably about 60% glycerol in distilled or deionized water. The resulting solution is mixed well, and from about 1.75 to about 3.0 parts by volume, preferably about 2 parts by volume, based on the above clear solution obtained prior to addition of the glycerol solution, of a 1.0 N solution of carbonate-free alkali metal hydroxide, preferably sodium hydroxide, in distilled or deionized water is carefully added to the above solution as an upper layer. One or more known standards (i.e. solutions containing known quantities of calcium ion) are similarly prepared according to the above process together with a bland (i.e., instead of blood serum or an equal quantity of a solution containing a known quantity of calcium ion, an equal quantity of distilled or deionized water is employed in the above process to form the blank). Whatever values are chosen within the above ranges, they must, of course, be identical for the standards and the blank. The above solutions are then mixed simultaneously and the colors allowed to develop undisturbed for five minutes. At the end of five minutes the absorbances of the unknown and the standards are read against the blank set at zero O.D. at 550 mμ wave length.

The quantity of calcium present in the unknown blood serum sample can readily be determined by simple standard calculations as shown hereinafter.

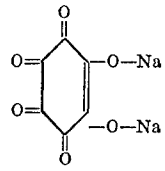
The invention will be better understood from a consideration of the following example which is given for illustration purposes only and is not meant to limit the invention.

EXAMPLE

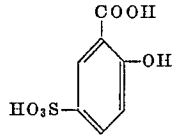
(a) Reagent A

Into a one (1) liter volumetric flask place: 500 mls.

of distilled or deionized water and 800 mg. of sodium rhodizonate.



Mix until the sodium rhodizonate is dissolved completely. Add 100.0 grams of sulfosalicyclic acid.



Mix until acid is dissolved completely and make up to volume with distilled or deionized water.

(b) Reagent B

60% solution of glycerol is distilled or deionized water.

(c) Reagent C

1.0 normal solution of sodium hydroxide in water, standardized, carbonate free.

Procedure

(1) Into three 13 x 100 mm. test tubes place:

(a) 3.0 mls. of Reagent A in each tube.

(b) 0.2 ml. of serum in the first, 0.2 ml. of standard in the second and 0.2 ml. of water (blank) in the third tube.

(2) Mix the contents of each tube well and centrifuge at 2,500 r.p.m. for 5.0 minutes.

(3) Into three 19 x 105 mm. test tubes place:

(a) 5.0 mls. of Reagent B in each tube.

(b) 1.0 ml. of supernatant liquid from the first 13 x 100 mm. tube in the first 19 x 105 mm. tube, 1.0 ml. of supernatant liquid from the second 13 x 100 mm. tube in the second 19 x 105 mm. tube and 1.0 ml. of supernatant liquid from the third 13 x 100 mm. tube in the third 19 x 105 mm. tube, and mix the contents of each tube well.

(c) Slant tubes, slowly and carefully overlay 2.0 mls. of Reagent C in each tube.

Note: At this state, the interphase between the two solutions in the three tubes should be colorless or slightly colored.

(4) Mix the contents of all three tubes as simultaneously as possible, and start stop watch.

(5) Allow color to develop undisturbed in each tube for a full five minutes.

(6) Immediately after the five-minute period is over, read the absorbances of unknown (first tube) and standard (second tube) against the reagent blank (third tube) set at zero O.D. at 550 mμ with a standard spectrophotometer.

Notes:

(1) Absorbances should be read within a period of ten minutes.

(2) Do not shake tube with color developed. Color tends to fade fast if shaken.

Calculation:

$$\frac{\text{O.D. of Unknown}}{\text{O.D. of Standard}} \times \text{Concentration of Standard} = \text{Concentration of Unknown}$$

Standardization:

Process for the preparation of a 20 mg. percent Ca stock standard

(1) Dry overnight pure calcium carbonate in an oven (300° C.).

(2) Since the molecular weight of $CaCo_3$ is 100, then the equivalent weight of $CaCo_3$ is 100/2 or 50.0.

(3) To make one liter of a standard which will contain 10 meq./L or 20 mg. percent Ca, weigh out $10 \times 50 = 500$ mg. of $CaCo_3$.

(4) Dissolve the $CaCo_3$ in 20 mls. of 1 N HCl in a beaker of 500 ml. capacity. Add 400 mls. of H_2O and read the pH (should be around 2.00).

(5) Very cautiously neutralize the solution using 1 N NaOH to pH 6.00. [+0.01]

(6) Transfer the resulting solution quantitatively to a one liter volumetric flask and q.s. to volume with deionized water.

Standardization: Plotting Curve.

5 mg. percent Ca Standard:

$5/20 = 0.25 \times 20 = 5$ mls. stock and 15 mls. water

10 mg. percent Ca Standard:

$10/20 = 0.5 \times 20 = 10$ mls. stock + 10 mls. water

15 mg. percent Ca Standard:

$15/20 = 0.75 \times 20 = 15$ mls. stock + 5 mls. water

20 mg. percent Ca Standard: Use straight stock standard.

Mix standards well and carry out the above procedure for calcium determination using the standards as unknowns. Plot optical density against concentration in mg. percent on graph paper. The resulting Cartesian graph will be a straight line with five reference points. This line obeys Beer-Lambert's law from the origin (zero O.D.) to 20 mg. percent. As an alternative procedure to that given, unknown serum samples can be measured using a blank, and the concentration of calcium in the serum read directly from the above graph.

What is claimed is:

1. A process for determining the amount of calcium ion in blood serum comprising the steps of:

(a) treating a measured quantity of blood serum with an aqueous solution containing from about 0.00136 to about 0.00184 parts by weight of sodium rhodizonate and from about 0.17 to about 0.23 part by weight of sulfosalicylic acid per part by weight of water, in amount sufficient to provide from about 0.0204 to about 0.0276 part by weight of sodium rhodizonate and from about 2.55 to about 3.45 parts by weight of sulfosalicylic acid per part by weight of blood serum,

(b) separating a clear solution from the resulting mixture,

(c) mixing said clear solution with from about 3 to about 13 parts by volume of about 40% to an about 75% solution of glycerol in water,

(d) mixing the resulting solution with aqueous alkali metal hydroxide,

(e) mixing the above solution thoroughly and allowing it to stand undisturbed for at least five minutes,

(f) measuring the intensity of the color at a wave length of 550 mμ in a spectrophotometer or colorimeter, and

(g) determining from the measurement the amount of calcium ion present in the blood serum; wherein in the above steps any water present is distilled or deionized water.

2. A process according to claim 1 wherein from about 0.00144 to about 0.00176 part by weight of sodium rhodizonate and from 0.18 to about 0.22 part by weight of sulfosalicylic acid are employed per part by weight of water, in amount sufficient to provide from about 0.0216 to about 0.0264 part by weight of sodium rhodizonate and from about 2.70 to about 3.30 parts by weight of sulfosalicylic acid per part by weight of blood serum, in step (a) and from about 7 to about 9 parts by volume of the aqueous solution of glycerol is employed in step (c).

3. A process according to claim 1 wherein the determi-

nation in step (g) is carried out by comparison with the color intensity of a known standard.

4. A process according to claim 1 wherein the sulfosalicylic acid in step (a) is in the form of a dihydrate.

5. A composition useful in the determination of the calcium ion content of blood serum comprising an aqueous solution of from about 0.00136 to about 0.00184 part by weight of sodium rhodizonate and from about 0.17 to about 0.23 part by weight of sulfosalicylic acid per part by weight of water; wherein said water is distilled or deionized water.

6. A composition according to claim 5 wherein from about 0.00144 to about 0.00176 part by weight of sodium rhodizonate and from about 0.18 to about 0.22 part by weight of sulfosalicylic acid are present per part by weight of water.

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U.S. Cl. X.R.

23—253; 252—408