United States Patent

Depositar et al.

[15] **3,663,175**

[45] **May 16, 1972**

[54] METHOD OF DETERMINING HEMOGLOBIN IN BLOOD

[72] Inventors: **Edward L. Depositar,** Indianapolis; **Robert K. Shipley,** Muncie; **David T. Santore,** Indianapolis, all of Ind.

[73] Assignee: **Bio-Dynamics, Inc.,** Indianapolis, Ind.

[22] Filed: **Oct. 5, 1970**

[21] Appl. No.: **78,159**

[52] **U.S. Cl.**.....**23/230 B,** 252/408

[51] **Int. Cl.**.....**G01n 33/16,** G01n 31/22, G01n 21/24

[58] **Field of Search**.....23/230; 252/408

[56] **References Cited**

UNITED STATES PATENTS

3,546,131	12/1970	Stern et al.	23/230 B
3,519,572	7/1970	Kita	23/230 B
3,427,135	2/1969	Pelavin et al	23/253 X
2,940,448	6/1960	Furlong, Jr.	23/253 X

Primary Examiner—Morris O. Wolk
Assistant Examiner—Elliott A. Katz
Attorney—Woodard, Weikart, Emhardt & Naughton

[57] **ABSTRACT**

A method of determining hemoglobin in blood using Drabkin's reagent wherein acetone is added to the reagent as a loss of color inhibitor and to aid in attaining immediate recovery of the peak absorbance of cyanmeth-hemoglobin.

5 Claims, No Drawings

METHOD OF DETERMINING HEMOGLOBIN IN BLOOD

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method of determining hemoglobin in blood using Drabkin's reagent. The reagent contains potassium ferricyanide ($K_3Fe(CN)_6$), potassium cyanide (KCN), and sodium bicarbonate ($NaHCO_3$). When the hemoglobin is added to this reagent, the potassium ferricyanide is reduced to potassium ferrocyanide ($K_4Fe(CN)_6$) and the hemoglobin is oxidized to methemoglobin which reacts with cyanide to produce to produce the stable chromogen, cyanmeth-hemoglobin.

2. Background of the Invention

This method was first described by Drabkin and Austin in the Journal of Biological Chemistry 98 719, (1932) and was discussed later by the same authors in the Journal of Biological Chemistry 112 51 (1935). This procedure is known as the cyanmeth-hemoglobin procedure. Because cyanmeth-hemoglobin is stable, the procedure is widely used in field tests for hemoglobin. The procedure lends itself to the preparation of Drabkin's reagent which can be shipped to the field for use by the physician or the clinical laboratory technicians in specially designed containers to simplify the hemoglobin test.

One of the problems with this procedure, however, is that the Drabkin's reagent is not stable to low temperature. It has been found that Drabkin's reagent, if exposed to freezing temperatures, loses its color with the subsequent loss of accuracy of the hemoglobin determinations made by using the reagent.

A typical Drabkin's reagent contains 61×10^{-5} moles of potassium ferricyanide, 77×10^{-5} moles of potassium cyanide, and 1190×10^{-5} moles of sodium bicarbonate per liter. This reagent is very stable. It has been reported in the article by Crosby and Houchin published in the Journal, Blood, 12 1136 (1957), that the cyanmeth-hemoglobin when formed in this reagent shows only a 4 percent loss of color when stored in sealed amber bottles for a period of 3 years.

The loss of color in this reagent on exposure to freezing temperatures is, of course, a major problem, particularly where the reagent is packaged and made available for field testing for hemoglobin.

SUMMARY OF THE INVENTION

In the preferred embodiment of the invention, the Drabkins reagent is made up and 0.2 to 10 weight percent of acetone is added as a loss of color inhibitor. The addition of acetone to the Drabkin's reagent also results in attaining immediate recovery of the peak absorbance of cyanmeth-hemoglobin in using the test.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The Drabkin's reagent is made up in the conventional manner. A typical formulation contains 200 milligrams potassium ferricyanide, 50 milligrams of KCN, and 1 gram of sodium bicarbonate per liter. The hemoglobin determination is made by the observance of the height of the 540 millimicron peak in a typical spectrophotometer scan of the solution containing the blood sample. The concentration is computed from the following equation:

$$C_u = A_u \times C_s/A_s$$

where C_u is the concentration of the unknown and C_s is the concentration of the standard, in grams per 100 ml. A_u is the absorbance of the unknown and A_s is the absorbance of the standard.

We have found that when the acetone is added to the Drabkin's reagent in the concentrations pointed out above, preferably at 0.6 percent, the freezing point of the Drabkin's solution is only slightly depressed. However, even though the reagent is frozen, the yellow color remains and there is no change in optical density of the reagent. This finding obviously solves the long standing problem caused by the loss of the color of Drabkin's reagent when exposed to freezing temperatures.

In addition, it was found that the addition of acetone to Drabkin's reagent obtained immediate recovery of the peak absorbance in cyanmeth-hemoglobin. This is particularly important in that the characteristic peak at 540 millimicrons is attained immediately with no initial peak at 570 millimicrons (the standard peak for oxy-hemoglobin). The precision of the test is improved since the instantaneous appearances cyanmeth-hemoglobin peak without the appearance of the oxy-hemoglobin peak eliminates any possibility of transposing the value of the oxy-hemoglobin reading with the resulting error in the calculation of the percentage hemoglobin in the blood sample.

Our invention is illustrated by the following specific but non-limiting examples.

EXAMPLE 1

Drabkins reagent was prepared by dissolving 200 milligrams of potassium ferricyanide, 50 milligrams of potassium cyanide, and 1 gram of sodium bicarbonate in a liter of water. The reagents were mixed thoroughly and when dissolved a quantity of acetone equal to 6 ml per liter of solution was added to one portion of this reagent. No acetone was added to the second portion. Both of the solutions were then exposed to the temperatures of −20° F for a period of about 96 hours. The solutions were then allowed to warm to room temperatures.

The test solutions were volumetrically dispensed in 13×50 mm. glass cuvettes known as Unitubes. All evaluations were performed using this container as the primary reaction cuvette. All cuvettes had a dispensed volume of 3.8 ml. of Drabkin's solution. All whole blood samples were 13 microliters delivered via the Bio-Dynamics Automatic pipette. The cuvettes were then transferred to a model 124 Coleman Spectrophotometer with a 2 millimeter slit. The spectrophotometer was operated at 10 mv full scale. Absorbances of the reagent were measured at the 540 millimicron peak. The height of the peak was essentially the same as the height of the peak using fresh Drabkins solution when the solution used was the solution containing acetone. In contrast, the height of the peak obtained when the solution used was the Drabkin's solution that had been frozen but contained no acetone was only about 30 percent of the height obtained from the same test using fresh Drabkin's reagent.

It is obvious from this data that the addition of acetone to the Drabkin's reagent provides an effective loss of color inhibitor for the reagent and allows the preparation of a reagent that can be transported without fear of loss of activity due to freezing.

EXAMPLE 2

In the process of evaluating the acetone modified reagent prepared according to the procedure described above, a spectral curve was run using a model 124 Coleman Spectrophotometer with a 2 millimeter slit. The spectrophotometer was operated at 10 millivolts full scale. Two separate tests were run. Blanks containing only the reagents were run as a reference. The first test was run using 3.8 ml of a Drabkins reagent that did not contain acetone. The second test was run using 3.8 ml. of Drabkin's reagent containing 6 ml of acetone per liter. Both of these tests were run with 13 microliters of blood having normal hemoglobin with a fast scan at 10 minutes to ascertain if secondary reactions have occurred.

The test run with the Drabkin's reagent that contained no acetone had two characteristic peaks, one appearing at 570 millimicrons and one at 540 millicrons. The 570 millimicron peak is, of course, associated with oxy-hemoglobin. When the same scan was run using the acetone modified Drabkin's reagent, the 540 millimicron peak was obtained immediately. There was no initial peak at 570 millimicrons. The reagent containing the acetone was then run using a 10 minute scan. There was no interference, primary or secondary.

It is obvious from these data that the Drabkins reagent containing acetone gives an instantaneous cyanmeth-hemoglobin

reading and eliminates the possibility of error to transposition of the oxy-hemoglobin reading from the spectrophotometer chart before the cyanmeth-hemoglobin appears.

What is claimed is:

1. In the determination the hemoglobin content of blood using the cyanmeth-hemoglobin method in which potassium cyanide, potassium ferricyanide and sodium bicarbonate are the active ingredients in the solution used to make the determination, the improvement comprising adding about 0.2 to 10 weight percent acetone to said solution to prevent loss of color in said solution on exposure of the solution to temperatures below 32° F and the resultant inaccuracies in the measurement.

2. The method according to claim 1 wherein the test solution contains about 61×10^{-5} moles of potassium ferricyanide, 77×10^{-5} moles of potassium cyanide and 1190×10^{-5} moles of sodium bicarbonate per liter.

3. The method of inhibiting loss of color in a hemoglobin test solution containing about 61×10^{-5} moles of potassium ferricyanide, 77×10^{-5} moles of potassium cyanide and $1{,}190 \times 10^{-5}$ moles of sodium bicarbonate per liter, on exposure of said solution to temperatures below 32° F which comprises adding 0.2 to 10 weight percent acetone to said solution.

4. In the determination of the hemoglobin content of blood using a solution containing potassium cyanide, potassium ferricyanide and sodium bicarbonate in which the potassium ferricyanide is reduced to potassium ferrocyanide the improvement comprising adding from 0.2 to 10 weight percent acetone to said solution to assure immediate attainment of the peak absorbance of cyanmeth-hemoglobin in the spectrophotometric determination of the hemoglobin of blood sample.

5. The method of immediately attaining the cyanmeth hemoglobin peak in the spectrophotometric determination of hemoglobin using the cyanmeth-hemoglobin procedure which comprises adding from about 2½ to 126 ml of acetone per liter to the reagent used in making the determination.

* * * * *