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METHOD FOR MEASURING THE GLUCOSE CONTENT OF BLOOD SERUM

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ABSTRACT OF THE DISCLOSURE

Method for the determination of glucose in blood serum, which may be used without deproteinizing said serum sample, comprising adding a small amount of iodine to the serum to oxidize free reducing agents in said serum, measuring the color, adding an oxidase reagent, and measuring the color change. PVP is used to intensify the colors thus produced.

This invention relates to a method for the analytical determination of the glucose content of blood serum or plasma.

A previously published process for determination of glucose in blood serum consists in adding to the serum sample a glucose oxidase, which in a properly buffered solution, breaks the glucose down to gluconic acid and hydrogen peroxide. Potassium iodide is then added to the mixture, and free iodine and potassium tri-iodide are produced, using ammonium molybdate as a catalyst. The iodine color is then measured by comparison with known color standards.

This process is described in "Clinical Chemistry, Principles and Technics," by Henry, published by Hoeber Medical Division of Harper & Roe-Publishers, 1964, at pages 632–635. It is also described in a publication by Malmstadt and Hadjiioannou, in Anal. Chem. 34, page 452 (1962).

It has been found that this method or similar methods using glucose oxidase cannot be applied directly to blood serum because these specimens are complex in nature and do not act as does pure glucose. Serum contains reducing agents of various kinds which take up the free iodine produced, thus resulting in an erroneously low analysis for glucose content. The greater portion of these reducing agents can be removed by precipitating the proteins present with cadmium or zinc hydroxide. The protein-free solution is then used for the analysis.

I have discovered a simple procedure which prevents the interference from reducing agents and allows direct analysis on serum or other body fluids without the protein precipitation step. The sample is pre-treated with a small amount of iodine for a short period prior to adding glucose oxidase. This iodine reacts with any reducing agent present and the result of this reaction may be determined easily by measuring the iodine color which remains. This measurement also includes any other interfering colored or turbid substances present in the sample such as bilirubin, hemoglobin, lipid or protein. Following this pretreatment with iodine, the glucose oxidase is added to release iodine, and the increase in color, which is directly proportional to the glucose, may be easily and accurately measured by visual or photometric means.

As an example, 0.05 ml. of the blood serum sample was treated with a standardized reagent containing potassium iodide, buffering salts to maintain the pH at approximate neutrality, and iodine. Five milliliters (5 ml.) of a reagent solution containing 1 gram by weight of potassium iodide, 0.002 gram of free iodine, 2 grams by weight of a buffer salt (K_2HPO_4/KH_2PO_4) to maintain the pH at 6.3 and 0.1 gram of ammonium molybdate for

each 100 ml. of aqueous solution was added to the sample. The mixture was allowed to incubate for 5 minutes, and the color measured by a photometer. Then 0.2 ml. of a concentrated solution of glucose oxidase were then added and incubated for ten minutes, following which the photometer reading for color concentration was again made. The increased concentration of yellow iodine color was then mathematically converted to concentration of glucose in the blood serum sample, using glucose comparison standards.

It has also been found that the color of the iodine in the solutions of the above described procedure may be intensified by as much as three times, by the addition of about 0.04 percent polyvinylpyrrolidone to the reagent solution specified above. The advantages of this modification are that a smaller sample, 0.02 ml. of blood serum, suffices for the analysis, the accuracy of analysis is improved, and the conformance to Beer's law is promoted. An excess of polyvinylpyrrolidone over the specified 0.04 percent does not improve the color intensification; and less than 0.04 percent results in a decreased intensification of color.

This liberation of iodine reaction can also be used to measure the glucose in other biological liquids, by making sure that the iodine present during the pretreatment is sufficient to oxidize all the reducing agents present. In other words, iodine color should remain after the pretreatment. The procedures as described above for blood serum may then be applied to the other biological liquids to obtain the glucose content.

The advantages will be apparent from the above description. The blood serum may be accurately analyzed by my improved method, without the necessity of using laborious steps to remove the proteins and other interfering ingredients before applying the steps of the colorimetric method above described.

I claim:

1. The method for the analysis of blood serum for the determination of the concentration of glucose in biological fluids comprising the steps of first adding to said serum a small but effective amount of free iodine, whereby to oxidize free reducing agents in said serum; measuring the color concentration after said oxidation reaction is completed; then adding an oxidase reagent comprising buffered glucose oxidase, potassium iodide, and catalyst ammonium molybdate, and measuring the increase in color concentration over the first color measurement.

2. The method for the analysis of blood serum for the determination of the concentration of glucose in biological fluids comprising the steps of first adding to said serum a reagent comprising buffering salts, potassium iodide, catalyst ammonium molybdate, and a small but effective amount of iodine whereby to oxidize free reducing agents in said serum; measuring the color of the resulting liquid after the reaction has been completed; then adding glucose oxidase; and measuring the increase in color concentration over the first color measurement.

3. The method defined in claim 1, wherein the oxidase reagent contains about 0.04 percent by weight of polyvinylpyrrolidone.

4. The method defined in claim 2, wherein the reagent contains about 0.04 percent by weight of polyvinylpyrrolidone.

5. A reagent solution effective for liberation of free iodine by glucose oxidase comprising glucose oxidase containing a proportion of a buffer salt

$$(K_2HPO_4/KH_2PO_4)$$

to maintain the pH of the reagent solution at 6.3, potassium iodide, a catalyst, and about 0.04 percent by weight of polyvinylpyrrolidone.

6. A reagent solution effective for liberation of free iodine by glucose oxidase comprising about one gram of potassium iodide, 0.002 gram of free iodine, 2 grams of a buffer salt (K_2HPO_4/KH_2PO_4) to maintain the pH at 6.3, 0.1 gram of ammonium molybdate, and 0.04 gram of polyvinylpyrrolidone, for each 100 ml. of aqueous solution.

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