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METHOD FOR DETERMINING GLUCOSE WITH o-TOLUIDINE REAGENT CONTAINING AN ARSENIC COMPOUND

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ABSTRACT OF THE DISCLOSURE

A diagnostic composition containing o-toluidine, thiourea and an oxide of arsenic is used for the quantitative determination of glucose in the body fluids of humans.

BACKGROUND OF THE INVENTION

This invention relates to a new and improved diagnostic composition which is useful for the quantitative determination of glucose in fluids, particularly body fluids such as urine, plasma, blood and the like.

The detection and quantitative determination of glucose in blood and urine is of great importance for diabetic patients whose diets must be controlled so as to regulate sugar intake and who must frequently be guided in this regard by regular checks on blood and urine glucose. Diabetes screening programs are dependent on the availability of rapid, inexpensive and accurate methods of glucose determination.

Procedures for the detection of sugar in blood and urine are well known in clinical chemistry. The Folin-Wu and Nelson-Somogyi procedures utilize copper reduction for the detection and determination of sugar. U.S. Pat. 3,008,879 describes a means for testing biological fluids for their glucose content which involves a combination comprising an enzyme system having glucose oxidase activity, a primary indicator material which is capable of being preferentially oxidized to a colored form in the presence of hydrogen peroxide and the material having peroxidative activity and, as a secondary indicator, a material which is capable of reducing the preferentially oxidized primary indicator material while simultaneously becoming colored.

The use of o-toluidine solutions in glacial acetic acid for the determination of aldosaccharides in serum or plasma is described by Hultman, E., Nature, 183, 108 (1959) and Dubowski, K. M. Clin. Chem. 8, 215 (1962). Ordinarily, glucose is the only aldohexose present in clinically significant amounts in blood serum; other aldohexoses that may be present in minute quantities are mannose and galactose. This test method is based on the formation of a colored complex of glucose and o-toluidine in glacial acetic acid. The concentration of this complex, and thus the intensity of the green color developed, is directly proportional to the concentration of glucose present in the reaction mixture, and can be measured photometrically.

For the determination of glucose in blood, the Folin-Wu and Nelson-Somogyi test methods can be applied only to protein-free filtrates of blood serum or plasma. There is some loss of specificity and reliability because of saccharoids and other reducing compounds that cannot be completely removed in preparation of a protein-free filtrate with resulting values higher than "true glucose" levels. Other disadvantages include the limited stability of the test reagents, the instability of the final test color, and critical factors in the test procedure involving temperature, heating time and alkalinity of reaction mixture.

The glucose-oxidase test is a highly specific test for glucose when correctly done with pure materials. A protein-free filtrate is required. Other disadvantages include

the sensitivity and limited stability of the test reagents, he necessary purity and standardization of the glucose-oxidase enzyme, the errors arising from interfering enzyme inhibitors, and the extreme care necessary during the three minute heating process where a matter of seconds can critically affect the accuracy of results.

The o-toluidine test is a simple, rapid, reliable and economical method for glucose determination. Importantly, there is no need for protein-free filtrates although the test can be performed with such filtrates. The test can be performed directly with blood serum or plasma. Orthotoluidine is the only reagent required. Acetic acid solutions are stable for at least six months. This stability is enhanced by the addition of thiourea. The final test color has good stability. An important disadvantage, however, of o-toluidine is its susceptibility to oxidation with its resultant effect on test sensitivity. The depressed sensitivity of the reagent results in a regression line with a lower slope when the optical densities of the final test colors are plotted against sugar concentrations, with serious loss of assay accuracy.

The object of this invention is to provide an improved diagnostic composition containing o-toluidine, thiourea and arsenic oxides for the simple, rapid, inexpensive and accurate quantitative determination of glucose in the biological fluids of humans.

SUMMARY OF THE INVENTION

This invention is concerned with a diagnostic composition containing o-toluidine for the quantitative determination of glucose in such biological fluids as blood and urine. It has been found that the incorporation of an oxide of arsenic in the o-toluidine reagent solution increases the sensitivity of the test reagent and increases the stability of the final test color in the assay procedure.

DETAILED DESCRIPTION OF THE INVENTION

The o-toluidine test method for the quantitative determination of glucose in blood and urine involves the formation of a colored complex of glucose and o-toluidine in glacial acetic acid which follows Beer's law. The green color, read at 630 nm., is stable (>97%) after standing one hour in air at 25° C.

o-Toluidine is relatively sensitive to oxidation. Bulk supplies of uniform quality and purity are difficult to obtain, with Eastman Chemical Company the preferred source. Freshly distilled o-toluidine is, of course, eminently suitable. The stability of o-toluidine solutions in glacial acetic acid (6.25% w./v.) is increased by the addition of thiourea (1.50% w./v.). The addition of an arsenate salt or oxide of arsenic increases the sensitivity of the o-toluidine reagent as reflected in the slope of the optical density curve of the final test colors.

The arsenic compound may be selected from the group consisting of the following:

sodium orthoarsenate: $Na_3AsO_4 \cdot 12H_2O$
sodium mono-H-orthoarsenate: $Na_2HAsO_4 \cdot 7H_2O$
sodium mono-H-orthoarsenate: $Na_2HAsO_4 \cdot 12H_2O$
sodium di-H-orthoarsenate: $NaH_2AsO_4 \cdot H_2O$
sodium metaarsenate: $NaAsO_3$
sodium arsenite: $NaAsO_2$
arsenic trioxide: As_2O_3
arsenolite: As_4O_6
claudetite: As_4O_6
arsenic pentoxide: As_2O_5
sodium thioarsenate: $Na_3AsS_4 \cdot 8H_2O$

The addition of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) to the o-toluidine reagent increases the sensitivity to glucose up to 120%. An increase in sensitivity, over solutions containing no additive, is observed even with small (<0.1% w./v.) concentrations of sodium arsenate additive. The

sensitivity increase levels off in the region of 0.5% w./v. additive, and finally attains a value of 120% increase at saturated levels of sodium arsenate (35% w./v.).

The color formed by the o-toluidine, with and without arsenate additive, is quite stable for the first hour. However, an increase in stability is noted for the sodium arsenate additive solution after 15 hours with a 32% decrease in color retained, compared to a 38% decrease for the control solution. This increased color stability is important when large numbers of test samples are run, and assay readings cannot be immediately made.

The following examples are provided to illustrate the present invention, but not to limit its scope.

EXAMPLE I

Preparation of reagent

Thiourea (0.3 g., 1.5% w./v.) is placed in a 500 ml. Erlenmeyer flask and dissolved in 150 ml. of fresh glacial acetic acid. Fresh opened o-toluidine from Eastman Chemical Company (12.5 ml., 6.25% w./v.) is added dropwise to the stirred acetic acid solution. Sodium mono-H-orthoarsenate, $Na_2HAsO_4 \cdot 7H_2O$, (100 mg., 0.5% w./v.) is added all at once and dissolved by gentle stirring under nitrogen. The solution is stored at room temperaure in a dark glass bottle under nitrogen.

Test procedure

(1) Using a suitable pipette add 0.05 ml. of blood serum or other sample to be tested to a 150 mm. x 15 mm., or similar, Pyrex test tube. If serum or plasma is used, deproteinization is not necessary.

(2) Add 4.0 ml. of o-toluidine reagent to the tube. Mix sample and reagent thoroughly, preferably with a vortex mixer.

(3) In a similar manner, prepare a reagent blank using 0.05 ml. of distilled water instead of sample, and prepare a glucose standard using 0.05 ml. of standard glucose solution instead of serum or other sample.

(4) Place the test tubes in a boiling water bath for exactly 10 minutes.

(5) Cool the solutions to room temperature, and determine optical densities at 630 nm.

(6) Determine the concentration of glucose in the sample from a previously prepared curve of optical density vs. glucose concentration, using a series of at least three different glucose concentrations in the range of expected results (50–400 mcgs. percent).

EXAMPLE II

The preparation of the o-toluidine test reagent of Example I is repeated in turn with sodium orthoarsenate ($Na_3AsO_4 \cdot 12H_2O$), sodium mono-H-orthoarsenate

($Na_2HAsO_4 \cdot 12H_2O$),

sodium di-R-orthoarsenate ($NaH_2AsO_4 \cdot H_2O$), sodium metaarsenate ($NaAsO_3$), sodium arsenite ($NaAsO_2$), arsenic trioxide (As_2O_3), arsenolite (As_4O_6), claudetite (As_4O_6), arsenic pentoxide (As_2O_5) and sodium thioarsenate ($Na_3AsS_4 \cdot 8H_2O$) in place of sodium mono-H-orthoarsenate ($Na_2HAsO_4 \cdot 7H_2O$), with comparable test results.

What is claimed is:

1. In the method for quantitatively determining glucose in the biological fluids of humans using a diagnostic composition containing o-toluidine, the improvement which comprises incorporating an arsenic compound selected from the group consisting of sodium orthoarsenate

($Na_3AsO_4 \cdot 12H_2O$)

sodium mono-H-orthoarsenate ($Na_2HAsO_4 \cdot 7H_2O$), sodium mono-H-orthoarsenate ($NaHAsO_4 \cdot 12H_2O$), sodium di-H-orthoarsenate ($NaH_2AsO_4 \cdot H_2O$), sodium metaarsenate ($NaAsO_3$), sodium arsenite ($NaAsO_2$), arsenic trioxide (As_2O_3), arsenolite (As_4O_6), claudetite (As_4O_6), arsenic pentoxide (As_2O_5) and sodium thioarsenate ($Na_3AsS_4 \cdot 8H_2O$).

2. The method of claim 1 wherein said arsenic compound is sodium mono-H-orthoarsenate

($Na_2HAsO_4 \cdot 7H_2O$).

3. A diagnostic composition for quantitatively determining glucose in the biological fluids of humans which consists essentially of a glacial acetic acid solution of o-toluidine, thiourea and an arsenic compound selected from the group consisting of sodium orthoarsenate

($Na_3AsO_4 \cdot 12H_2O$),

sodium mono-H-orthoarsenate ($Na_2HAsO_4 \cdot 7H_2O$), sodium mono-H-orthoarsenate ($Na_2HAsO_4 \cdot 12H_2O$), sodium di-H-orthoarsenate ($NaH_2O_4 \cdot H_2O$), sodium metaarsenate ($NaAsO_3$), sodium arsenite ($NaAsO_2$), arsenic trioxide (As_2O_3), arsenolite (As_4O_6), claudetite (As_4O_6), arsenic pentoxide (As_2O_5) and sodium thioarsenate

($Na_3AsS_4 \cdot 8H_2O$).

4. The diagnostic composition of claim 3 wherein said arsenic compound is sodium mono-H-orthoarsenate ($Na_2HAsO_4 \cdot 7H_2O$).

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