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TEST COMPOSITION AND METHOD FOR DETECTING REDUCING SUGARS IN AQUEOUS FLUIDS

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ABSTRACT OF THE DISCLOSURE

Test composition and method for detecting reducing sugars in aqueous biochemical and industrial fluids comprising a dry solid mixture of an acid material, an alkaline material, a cupric salt and a surface active agent capable of forming a substantially monomolecular film at the liquid-vapor interphase of the fluid being tested.

BACKGROUND OF THE INVENTION

The estimation of glucose in urine is at present the best practical means available to the physician, as well as to the diabetic patient, for evaluating and controlling the intake of sugars, effectiveness of antidiabetic medicaments, and the general condition of the individual suffering from diabetes mellitus. In order to be effective, such sugar tests must be simple, rapid, capable of being performed daily by non-technical personnel and must be reasonably quantitative over a range of pathological urine glucose concentrations.

In addition to clinical testing, there is a need in industry, and particularly in food and beverage processing industries, for a glucose or reducing sugar test having the above-noted capabilities. For example, a quantitative determination of sugar in wine can be used as a means for evaluating its "dryness." The disappearance or absence of reducing sugars in beer can be used as a means for determining the progress or completion of the fermentation process. Still another example of the need for a quick and quantitative sugar test can be found in the potato chip processing industry where too much sugar in the raw potatoes results in a charred or brown chip.

DESCRIPTION OF THE PRIOR ART

Methods heretofore available for detecting reducing sugars in urine were based on the principle of heating a mixture of measured aliquots of urine and an alkaline copper solution (such as Benedict's, Fehling's, Folin-McElroy) or an alkaline bismuth solution (such as Nylander, Alment). In qualitative estimations, the precipitation of cuprous oxide from the cupric compound, or the reduction of the bismuth ion to black metallic bismuth, indicates the presence of a reducing carbohydrate.

In quantitative urine sugar estimations, the usual method employed heretofore has been that of Benedict (Journal of the American Medical Association, vol. 57, page 1193, 1911). This involves adding measured amounts of urine to an aliquot of a standard solution, while boiling the latter, until the blue cupric compound contained therein is completely decolorized, forming cuprous thiocyanate. By a calculation, the percentage of reducing substance in the urine is determined.

Sheftel, U.S. Pat. No. 1,769,862, has described a method for the quantitative determination of sugar in urine based on the following steps: (a) Measured aliquots of a special modified Benedict solution containing creatinine and a hydrophilic colloid are mixed with a measured aliquot of urine; (b) the mixture is heated in boiling water for five minutes; (c) if a change in the original blue color appears, the solution is compared with a color scale and the corresponding percentage of sugar is estimated.

All of the procedures thus far mentioned have one great drawback. They all require an extraneous source of heat. This drawback was solved by the method described in U.S. Reissue Pat. No. 23,705 which discloses the use of a tablet test composition containing a cupric salt and acid material and an excess of an alkaline material such that when the tablet is added to an aqueous fluid containing reducing sugars, the exothermic neutralization reaction between the acid and base creates sufficient heat to enable the cupric salt to be reduced to cuprous oxide. This is, of course, accomplished without recourse to any extraneous source of heat. Moreover, the alkaline material is present in excess of the amount required in the neutralization reaction so that the reduction of the cupric salt may proceed in an alkaline pH environment.

Over the years it has been found that the use of this tablet test system in fluids containing certain foam-forming or gelling constituents often results in the following adverse test reaction conditions:

(1) The boiling (heating) time of the fluid may be increased as much as five times that normally required.
(2) The test reaction mixture may lift the tablet at least partially out of the solution, thereby resulting in insufficient heating of the test mixture.
(3) Color changes may be obscured to the extent that the end point is not readily visible.
(4) The reaction may proceed in a manner such that proper heat dissipation is not achieved, thereby causing erroneous results.
(5) Certain pathological constituents in the test fluid and particularly in urine, may be coagulated by the exothermic test reaction and cause an uneven color suspension which makes the reading thereof difficult.

Certain of the above adverse reactions in testing urine for glucose have in the past been eliminated by the addition thereto of materials such as caprylic alcohol. Such additions have, however, been made as a sequential step after the test procedure has commenced, and the materials suggested have been liquids or solutions which require the use of additional measuring and dispensing equipment, reagent solutions and so forth.

OBJECTS OF THE INVENTION

It is therefore an object of the present invention to provide a single entity test composition of the type described herein which provides uniform results regardless of the presence of constituents other than reducing sugars in the fluid being tested.

Other more specific objects are the elimination of the adverse test reaction conditions enumerated hereinabove.

SUMMARY OF THE INVENTION

It has now been found that an improved single unit test composition for detecting reducing sugars in aqueous fluids containing ingredients which have heretofore caused the aforesaid adverse test reaction conditions is provided by a combination of (a) a cupric salt, (b) an acid material, (c) an alkaline material or mixture of alkaline materials and (d) a surface active agent which tends to form a continuous substantially monomolecular film at the liquid-vapor interphase of the fluid being tested, said surface active agent being uniformly present in the test composition on a solid, particulate carrier material therefor.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The basis of all test systems containing alkaline cupric ions heretofore employed is: (a) a cupric salt, such as cupric sulfate; (b) an alkaline-reacting substance, such as sodium carbonate, and/or potassium hydroxide; and, (c) a member of the group of salts that are capable of

holding cupric hydroxide in solution. Fehling (Annalen, vol. 72, page 106, of 1849) used normal alkali-metal tartrates (Rochelle salts), while Benedict (J. Biol. Chem. vol. 3, page 101, of 1907) used normal alkali-metal citrates and Folin-McEllroy (J. Biol. Chem. vol. 33 page 513, of 1918) used normal alkali-metal phosphates.

Stable test compositions of excellent specificity for reducing carbohydrates, and fulfilling the objects of the present invention, may be obtained by preparing solid, dry mixtures of the following ingredients in proper proportions:

(a) A water soluble cupric salt;

(b) A solid alkali material of the group consisting of the oxides and hydroxides of the alkali metals;

(c) A member of the group of solid acids and acid reacting salts forming water-soluble complexes with cupric ions in alkaline solution, which group consists of citric acid, tartaric acid, the alkali metal acid salts of citric acid; and,

(d) A surface active agent (as will be described hereinafter) and a solid, particulate carrier therefor.

Preferably, for convenience of the user, the dry mixtures are tableted so that each tablet will be sufficient to make one analytical determination. When these compositions, either in powder or tablet form, are mixed with a small amount of an aqueous specimen such as urine, they dissolve rapidly with the evolution of a considerable amount of heat. It may also be desirable to dilute the test solution with water. In the absence of a reducing carbohydrate, a clear blue solution is obtained. In the presence of a reducing sugar, a green, yellow or brownish red precipitate of cuprous oxide is obtained, which is held in suspension by the highly concentrated alkaline solution. To determine quantitatively the amount of reducing carbohydrate present in the test specimen, the color of the suspension may be compared with panels of a standard color chart for known concentrations of glucose (or other reducing carbohydrates) in urine (or any other specimen being tested).

In accordance with Beer's law, on which the entire art of colorimetry is based, the intensity of the observed color of the precipitate is directly proportional to the concentration of the colored substance (i.e., cuprous oxide). However, since the concentration of the cuprous oxide is also directly proportional to the concentration of reducing carbohydrates originally present in the specimen, the observed color is likewise directly proportional to the concentration of the reducing substance.

The surface active agents of the present test composition are those substances which when intermixed with the test composition and added to the fluid being tested tend to form inelastic substantially monomolecular films at the liquid-vapor interphase of the fluid being tested for reducing sugars. Such materials include, inter alia, high molecular weight diamides or polyamides of substances such as the lower aliphatic and aromatic diamines or polyamines, castor oil, polar vegetable oils, polyethenoxy esters and ethers, polyglycols, higher molecular weight sulfonamides and disulfonamides, imidazolines of high molecular weight, silicone oils, fatty alcohols of medium molecular weight, lower phosphate esters such as tributyl phosphate, beeswax, cellulose ethers and microcrystalline wax. It will be appreciated that certain of these surface active substances are solids at room temperature and in order that they may be use as described hereinbelow, they must be dissolved in a suitable organic solvent system.

It has unexpectedly been found that although such materials can be used alone as a sequential addition to the test system, in order to incorporate same directly into a test composition, a carrier plus the surface active agent must be used. Such carriers are solid, inert substances upon or in which the surface active agent is uniformly dispersed or admixed. Exemplary of such carriers are, sodium silicate, aluminum silicate, infusorial earth, talcum powder, titanium dioxide and kaolin. The preferable carriers are the silicates, such as sodium silicate, magnesium silicate, aluminum silicate and mixtures thereof.

The surface active agents are incorporated with the carrier prior to admixing same with the test composition, by simply grinding together in a mortar, blending, or other means of admixing ingredients. If the surface active agent is a liquid or oil, it may be dissolved in a solvent, added to the inert carrier and the solvent evaporated to leave an even dispersal of the surface active agent on or in the carrier.

Referring now to concentrations of surface active agents and the carrier thereof, it has been found that a wide range of concentration may be utilized, depending upon the type and activity of the surface active constituent being used. Generally speaking, however, from about .01% by weight to about 1.0% by weight of surface active agent is used in a test composition while the ratio of surface active agent to carrier may be adjusted to give the maximum amount of surface active agent per unit weight of carrier and still have a free flowing powder. Generally speaking the ratio of surface active agent to carrier may be from about 1:60 to 0.6:1.

If desired, dilutents, excipients and ancillary or auxiliary agents may be incorporated into the dry compositions. Thus, a minor proportion of an alkali metal bicarbonate or carbonate (e.g. sodium bicarbonate or carbonate) may be added. When the dry composition containing such a material is added to the liquid specimen being tested, the solid acid or acid reacting salt therein will interact with the alkali metal bicarbonate or carbonate liberating gaseous carbon dioxide. The effervescence thus produced serves to accelerate the mechanical disintegration of the composition, especially if it is in tablet form. The reaction is thus speeded up, and the determination is effected more promptly. Since the total heat evolution is thereby effected within a shorter period, there is less loss of heat by radiation, conduction and vaporization, and traces of reducing carbohydrates are detectable, which might otherwise go undetected.

A desiccating agent, such as calcium chloride, and diluents and excipients may be employed without adversely affecting the sensitivity and specificity of the compositions. Auxiliary agents, such as creatinine and the hydrophilic colloids, may be incorporated, their presence in the test solution being advised by Sheftel, to prevent the formation of the red form of cuprous oxide.

Typical examples of ingredients from group (a) above that may be used (i.e. water soluble cupric salts) in preparing the compositions are cupric sulphate, cupric chloride, and cupric acetate.

Typical examples of members of group (b) (i.e. oxides and hydroxides of alkaline metals) that may be used in the diagnostic compositions are sodium hydroxide and potassium hydroxide.

Typical examples of ingredients of group (c) (i.e. solid acids and acid reacting salts which form water soluble complexes with cupric ions in alkaline solution) that may be used in preparing the dry diagnostic composition are: citric acid (monohydrate) and, tartaric acid.

Because of the hygroscopicity of compositions within the scope of this invention, it is desirable, although by no means imperative, to use anhydrous reagents instead of the corresponding hydrates. Thus anhydrous cupric sulfate yields more stable compositions than the hydrate salt and this greater stability holds for all of the ingredients having two forms of high and low (or no) water content. However, if a hydrate does not have a tendency to deliquesce at the temperature encountered in the localities where these compositions are to be used, it may be used in a preparation of the present invention.

The heat evolved during the reaction of the test compositions of this invention with urine, or similar specimens, is predominantly due to two exothermic reactions. One of these is the heat of solution of the alkali metal

oxide, or hydroxide, in the aqueous fluid. The other is the heat of neutralization of the solid acid or acid reacting salt with the alkali metal hydroxide. The heat evolved will suffice to effect the reduction of the cupric complex to cuprous oxide, which forms a precipitate of characteristic color.

The dry compositions may also be used for the qualitative detection and quantitative estimation of reducing carbohydrates other than glucose. Thus, they may be used to detect pentosuria, to estimate the amount of galactose in urine during a galactose tolerance test for hepatic function or hyperthyroidism, or the amount of lactose in milk.

As used and claimed herein, the term detecting or detection means both the qualitative and quantitative estimations of reducing sugar in the fluid being tested.

EXAMPLE 1

Preparation of test reagent composition

A basic test composition formulation was made to contain the following ingredients and quantities thereof per test unit:

	Mg.
Copper sulfate	19.35
Sodium hydroxide	232.5
Citric acid	200.0
Sodium carbonate	80.0

A second mix was then prepared by adding 15.0 ml. of n-octyl alcohol to 20.0 grams of sodium silicate in a mortar and grinding with a pestle until the alcohol was evenly dispersed in or on the silicate. The result was a fine, particulate admixture of surface active agent and carrier therefor. The second mix was then added to and mixed well with the basic test composition in a weight ratio of 1 mg. of second mix per test unit (532 mg.) of basic mix. The admixture was then formed into tablets, each tablet having a test unit quantity of ingredients. Other admixtures of n-octyl alcohol-sodium silicate were prepared and added to the basic test composition at a rate of from about 0.25 mg. to about 3 mg. per test unit. The preferable range for this admixture was found to be 0.3 mg. to 1.5 mg. per test unit.

EXAMPLE 2

Example 1 was repeated except that silicone oil (dimethyl polysiloxane having a viscosity equal to about 60,000 centistokes at 25° C.) was substituted for the n-octyl alcohol. Optimum concentration of silicone oil-sodium silicate was found to be from about 0.5 mg. to about 10 mg. per test unit.

EXAMPLE 3

Example 1 was repeated except that talcum powder was substituted for the sodium silicate.

EXAMPLE 4

Comparison of present test compositions to prior art test compositions

Two drops of urine containing 300 mg. added protein (bovine serum albumin) and 1% added glucose and 10 drops of distilled water were added to each of two small test tablets labeled No. 1 and 2. Two drops of the same urine sample but without the added protein and 10 drops of distilled water were added to a third test tube labeled No. 3 A tablet prepared as described in Example 1 was added to the No. 1 tube and tablets prepared as in Example 1 but without the 1 mg. of surface active agent and carrier were added to each of the tubes numbered 2 and 3. The boiling times for the tubes numbered 1 and 3 were substantially the same (20 seconds) while the boiling time for tube No. 2 was fifty-five seconds longer (75 seconds). Moreover the tablet in test No. 2 tended to lift out of the tube while the boiling of tube Nos. 1 and 3 was even and controlled. Thus, the use of a surface active agent in a test reaction system containing a pathological constitutent (protein) resulted in a normal boiling time, whereas when the surface active agent is not present, the boiling time is three to four times longer than normal.

What is claimed is:

1. In a test composition in dry solid form for detecting reducing sugars in aqueous solution, said composition utilizing a mixture of a water soluble solid cupric salt, a solid acidic material, and a quantity of solid alkaline material in excess of that required to neutralize the acidic material in an amount adequate to generate during such neutralization sufficient heat for the conversion of the cupric salt to cuprous oxide by the action of any reducing sugar present, the improvement which comprises the inclusion in the test composition of a surface active agent which tends to form an inelastic substantially monomolecular film at the liquid-vapor interphase of the fluid being tested for reducing sugars, said surface active agent being dispersed in said test composition in admixture with a solid carrier material therefor.

2. The test composition of claim 1 wherein the alkaline material is an alkali metal hydroxide.

3. The test composition of claim 1 wherein the alkaline material is a mixture of an alkali metal hydroxide and an alkali metal carbonate.

4. The test composition of claim 1 wherein the acid material is selected from the group consisting of citric acid and tartaric acid.

5. The test composition of claim 1 wherein the cupric salt is cupric sulfate.

6. The test composition of claim 1 wherein the surface active agent is selected from the groups consisting of high molecular weight diamides and polyamides, castor oil, polar vegetable oils, polyethenoxy esters and ethers, polyglycols, high molecular weight sulfonamides and disulfonamides, imidazolines of high molecular weight, silicone oils, fatty alcohols at medium molecular weight, lower phosphate esters, beeswax, cellulose ethers and microcrystalline wax.

7. The test composition of claim 1 wherein the solid carrier is selected from the group consisting of sodium silicate, aluminum silicate, infusorial earth, talcum powder, titanium dioxide and kaolin.

8. The test composition of claim 1 wherein the surface active agent is n-octyl alcohol and the carrier is sodium silicate.

References Cited

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,630,958 Dated December 28, 1971

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It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1 - line 43	Seventh word should read -- browned -- rather than "brown"
Column 3 - line 65	Ninth word should read -- used -- rather than "use"
Column 4 - line 13	Fifth word should read -- therefor -- rather than "thereof"
Column 4 - line 24	Third word should read -- diluents -- rather than "dilutents"
Column 5 - line 60	Insert -- % -- immediately following 300 mg.
Column 5 - line 66	Insert -- . -- immediately following No. 3

Signed and sealed this 27th day of June 1972.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

ROBERT GOTTSCHALK
Commissioner of Patents

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