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DIAGNOSTIC INDICATOR

Frank E. Hammer, Chicago, and Don Scott, Skokie, Ill., assignors, by mesne assignments, to G. D. Searle & Company, Chicago, Ill., a corporation of Delaware
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This invention relates to a method of testing for glucose. More particularly, it relates to a product for determination of materials in biological and industrial fluids which can be acted upon by enzymes in the presence of oxygen or air. Still more particularly, it relates to a product for quick determination of glucose content of blood, urine, and starch hydrolysates.

The present invention relates to material for quantitative determination of, for example, sugars comprising a polyglycol composition with an oxidase enzyme capable of catalyzing a reaction when a sugar and air are present and a water-soluble indicating substance capable of producing a color change in the presence of a product of the enzyme reaction.

Numerous methods are known which can be used to measure or to estimate the amount of, for example, glucose in fluids such as urine and blood. The more widely used of these conventional methods are based on the use of alkaline copper solutions which are heated with the material to be tested to precipitate cuprous oxide. Another popular method is the use of ferricyanide.

These conventional methods have the disadvantage of not being specific for glucose in that they determine reducing substances, generally calculated as glucose.

More recently, glucose detecting compositions containing an enzyme which acts on glucose to produce a reaction product whose presence is readily demonstrable, have been introduced. Compositions of this type composed, for example, of glucose oxidase, peroxidase and indicators which undergo color reactions, are available commercially.

The enzymatic determinations of glucose mentioned hereinbefore have had the drawback of requiring the use of large amounts by volume of diagnostic solution with the consequent use of large amounts of relatively expensive enzymes. In direct contrast, the unique compositions of this invention permit accurate determination of sugars at substantially lower enzyme levels, irrespective of the volumes of reactants.

In practicing the instant invention, a solution is prepared containing oxidase enzyme, peroxidase enzyme, a polyglycol, a chromogen and, if desired, a buffer for the solution. Such a solution may be a polyglycol solution of a chromogen containing the other components in dispersed solid form, or it may be an aqueous solution which should be promptly frozen and lyophilized if it is not going to be used immediately so as to avoid developments which will alter the chromogen and might result in insolubilization of the chromogen.

In a preferred embodiment of the invention which lends itself to easier processing of products to be stored, a solution is prepared containing oxidase enzyme, peroxidase enzyme, a polyglycol and a buffer capable of maintaining the solution at a pH in the range between about 4 to about 7.5. In this preferred embodiment, the chromogen is incorporated in a separate solution. This solution contains a polyglycol and a chromogenic hydrogen donor which is oxidized by the products of the reaction of the oxidase enzyme.

These solutions, which are combinable due to the presence of the polyglycol, form a homogeneous solution which will, upon contact with a glucose-containing solution, give a distinctive color shift measurable in nu-

merous ways such as, by a spectrophotometer, colorimeter, comparator block, etc. In the preferred embodiment of this invention, to insure against any premature reactions due to impurities, etc., the polyglycol-containing solution of the oxidase enzyme and of the chromogen are kept separate. The polyglycols are a common menstruum which permits reconstituting the separated materials with water and then combining them to form a homogeneous solution. The phenomenon of using the polyglycol as a constituent of the mix is unique. Polyglycols render the chromogens more readily soluble. In addition, the polyglycols are inert to the oxidation that causes the color shift, whereas aliphatic alcohols added to enhance chromogen solubility may be oxidized in preference to the chromogen interfering with the accuracy of diagnostic tests. Thus, the solutions of polyglycols and chromogen are novel products for use in conjunction with any test composition which produces hydrogen peroxide or oxidizing conditions of sufficient strength to effect a chromogen color shift. When the polyglycol solutions are frozen and lyophilized, they can be redissolved in water to form homogeneous solutions, whereas simple aqueous solutions of oxidase enzymes and of chromogen treated in the same manner as the polyglycol solutions produce a precipitate or turbidity which drastically interferes with the color response in use of the resultant solution as a test indicator.

In the preferred method of preparing a diagnostic indicator capable of testing for glucose in amounts, for example, up to about 0.2 mg. glucose per milliliter of reconstituted indicator, in the form of a storable article of commerce, an aqueous solution containing 1 to 40 units per ml. of oxidase enzyme is prepared. This solution also contains peroxidase enzyme, the polyglycol and a buffer such as a citrate or phosphate buffer. A separate solution, if prepared, contains a polyglycol and from 0.15 to 8 milligrams per ml. of chromogen. In general, the high concentrations are preferred. The concentrated chromogen solutions may be water-free, or may be so concentrated as to permit the introduction of the necessary ingredients in a smaller amount of water, facilitating lyophilization.

One of these solutions is introduced into a storage container which may be a vial designed to hold sufficient material for a single test or for a larger number, such as thirty tests, or may be introduced into larger containers, such as tubes, flasks, and the like. The solution first introduced into the container is cooled to solidify it. The second solution, either in the form of a pre-solidified mass or as a liquid is introduced into the same container. If the latter solution is in liquid form, the container and its contents are subjected to low temperatures to cool the second solution to a solid mass.

When the container holds only solid material, the contents of the container are lyophilized. The container may then be sealed and stored. Refrigeration during storage is conducive to a longer shelf life, though room temperature may be used for limited storage times.

The diagnostic test requires that the lyophilized solid materials be reconstituted with a proper quantity of water prior to use. When the composition produced by reconstituting the polyglycol compositions with water, is contacted with a glucose-containing fluid, the chromogen is altered to produce a color shift. The color of each of the glucose solutions under test is correlated to control samples having known concentrations of glucose.

To be an effective diagnostic indicator, a test must be capable of developing a uniformity of color over, for example, a test range for glucose of up to 0.2 milligram per ml. of reconstituted diagnostic indicator, i.e., the result of plotting absorbancy versus milligrams of glucose should be a reproducible relationship, preferably a

straight line. It is possible using amounts of oxidase enzyme small enough to be economically significant, for example, 1.6 units of glucose oxidase per cc. of test aliquot to achieve this linearity of the plot of absorbancy versus milligrams of glucose. The linearity is achieved by maintaining a minimum ratio in milligrams of chromogen to units of glucose oxidase of approximately 1 to 10, usually a ratio in the range between 1.1 to 10 and 1.5 to 10 and achieving a complete solubility of the necessary amounts of chromogen by incorporating the polyglycols.

While the ratio set forth is a preferred embodiment, other ratios are usable. For example, if the reaction time for forming color is to be shortened, the ratio may be reduced by increasing the amount of glucose oxidase. Similarly, if catalase is present in significant amounts, a higher level of glucose oxidase may be used to offset this diversion. It will be understood, therefore, that the ratio of chromogen to enzyme of 1 to 10 is an approximately minimum ratio and variations in the compositions generally will require increases in the quantity of glucose oxidase.

The polyglycols useful for the purposes of this invention should be free of anti-oxidants to insure adequate response at very low levels of glucose. The conventional polyglycols of commerce are not useful for the purpose of this invention because they contain amounts of anti-oxidants which may interfere with the chromogen color change reaction. The polyglycols preferred for the purposes are polyethylene glycol and related compounds that are normally solid at about 40° C. (104° F.) and are substantially soluble in water at temperatures above about 20° C. (68° F.). However, chromogen solutions may be prepared without aqueous media, by dissolving in polyethylene glycols which have been heated to melting temperatures, or are liquid at about 40° C. (104° F.).

In the article of commerce prepared according to this invention, the presence of the polyglycol in the solutions has a unique effect. The polyglycol solutions, when reconstituted with water, are substantially free of the turbidity encountered when lyophilized aqueous materials free of polyglycols are reconstituted, which turbidity renders glucose tests at best difficult to read and for most purposes reduces the available chromogen so as to be useless as a quantitative diagnostic tool. While incorporation of polyglycol into the enzyme solution is not essential, it is preferred. The polyglycol tends to make the freezing point of the aqueous enzyme solution and an aqueous chromogen solution substantially the same and to facilitate the mixing of the enzyme solution and chromogen solution at the time of resolution in water. Another advantage of the use of polyglycol in the enzyme solution is that it permits introduction of a greater total amount of polyglycol than can be incorporated in the chromogen solution alone. Polyglycol is useful in amounts in the total solution of up to about 10%. This is a critical upper limit of amount by weight of glucose solution in the final solution. Polyethylene glycol, for example, in amounts in excess of about 10% inhibits reaction and makes accurate determination of glucose substantially impossible.

The nature and extent of color development may vary depending upon the kind and amount of chromogen which is utilized. Nevertheless, when a number of test units are made up to the same predetermined specifications, for comparison with known solutions, the amount and nature of the color development responsive to the amount of glucose present in the solution to be tested, provides a quantitative glucose determination.

More in detail, in practicing this invention adaptable to testing for materials which can be reacted with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide, for example, carbohydrates, particularly saccharides and more particularly sugars and amino acids, in its preferred form adapted to test for glucose, a first solution is prepared comprising as fol-

lows: 15 to 40 units per ml. of oxidase enzyme, 100 to 250 mg. per ml. of buffer salt, 0.05 to 0.1 mg. per ml. of peroxidase, 3.5 to 100 mg. per ml. of polyglycol. A separate solution is prepared comprising as follows: 3 to 8 mg. per ml. of chromogen, 100 to 200 mg. per ml. of polyglycol.

In the embodiment of the invention intended for the testing of glucose, an enzyme system containing glucose oxidase with or without catalase, preferably substantially free of catalase, is used. However, other oxidase and dehydrogenase systems that are capable of catalyzing a reaction in an aqueous medium between molecular oxygen and a specific substrate for an oxidase or dehydrogenases and result in the formation of hydrogen peroxide, may be employed. Thus, molecular oxygen will combine with galactose in the presence of galactose oxidase, L or D amino acids in the presence of their specific oxidases, succinate in the presence of a dehydrogenase system consisting of succinic dehydrogenase, DPN, FAD, cytochrome and cytochrome oxidase.

To be an effective diagnostic material, the oxidase containing solution should be buffered to provide a pH in the range between about 4 and about 7.5. Citrate salts, such as sodium citrate, citric acid, potassium citrate, etc., phosphate salts, such as mono- and disodium phosphates, potassium acid phosphate, etc., are useful buffers when suitably mixed or adjusted in pH by the use of KOH, NaOH, HCl, in combination with one or more of the above. It will be recognized that different enzymes vary in their operable pH range. For example galactose oxidase shows good operating characteristics at a pH between about 5.5 and about 7.0.

The preferred water-soluble chromogen indicator component of the test compositions is orthodiansidine dihydrochloride. Other indicators which may be used include o-tolidine, gum guaiac, o-cresol, an iodide salt and the like.

Peroxidase for use in these compositions may be obtained from horseradish. Instead of horseradish peroxidase, a compound of similar peroxidative activity, such as the hemoglobins of blood, may be substituted.

The following examples of specific embodiments of the invention are given by way of illustration and without any intention that the invention be limited thereto. It will be recognized that such variables as total reaction volume and temperature and time of incubation are subject to considerable variation. For example, the total reaction volume can be reduced for individual tests to approximately 0.5 cc. while retaining the same amounts of reacting ingredients on a dry basis, i.e., attain higher concentration of reactants for smaller reaction volumes. Time and temperature for incubation can also be varied from 30° C. for 30 minutes provided the combined effect of temperature and the holding time is not deleterious to the reaction.

Further, dissolving of the article of commerce need not be in water, for individual test compositions may be dissolved in an aqueous unknown provided volume relationships are taken into account.

EXAMPLE I

A glucose oxidase containing composition designated solution A was prepared by mixing together: 200 mg. of glucose oxidase (24,000 units total), 748 ml. of citrate buffer (1 molar solution-pH 5.0), 3.8 ml. of peroxidase (10 mg. per ml.) and 50 grams of polyethyleneglycol (4000). This composition was made up to a solution having a volume of one liter.

A chromogen-containing solution designated solution B was prepared by mixing together: 2.5 grams of ortho diansidine dihydrochloride, 62.5 grams of polyethyleneglycol (4000), 250 ml. of water. This solution B was made up to a total of 500 ml. with distilled water.

In preparing the article of commerce, 2 ml. of solution A was introduced into a 30 ml. vial and the solution frozen

by holding in a refrigerator maintaining a temperature of about plus 10° F. When solution A was a solid, 1 ml. of solution B was introduced into the vial and the contents frozen by again subjecting them to a temperature of about plus 10° F.

The vial was next subjected to freeze drying, i.e., lyophilized to produce substantially water-free solids in the vial.

In adapting the above article of commerce for use, water was added to the vial so that a 30 ml. solution was produced. In order to test for glucose in a corn syrup (43° Bx.), 2 ml. of diluted corn syrup having a total glucose content of 0.1 milligram was mixed with 1 ml. of the test solution.

After thorough mixing and holding at 30° C. (86° F.) for thirty minutes to allow time for reaction, 10 ml. of 6 normal sulfuric acid was added to the reacting mixture to arrest the reaction, this amount of sulfuric acid being sufficient to raise the normality of the total mass under test to a normality in excess of 4 normal. At this point, the solution acquired a pink color when measured in a spectrophotometer using light of a wave length of 5400 Angstrom units showing the presence of 16.1% glucose in the undiluted corn syrup.

The above test for glucose utilized specific aliquot sizes of solution to be tested for glucose and of test solution. It will be understood that both reactants may be diluted to concentrations different from those set forth and used in quantities different from those set forth. For example, 4 ml. of the diluted corn syrup of Example I could be mixed with 2 ml. of test solution to produce 6 ml. total of reaction mixture. In addition, the test solution could be diluted with water to give greater or lesser concentrations of glucose oxidase provided the contents of the vial of, by way of illustration, Example I is made to not less than 4 ml. to insure the avoidance of precipitation of chromogen.

EXAMPLE II

A glucose oxidase containing composition designated A was prepared by mixing together 6.15 ml. of glucose oxidase (6150 units total), 187 ml. of citrate buffer (1 molar solution-pH 5.0), 0.93 ml. of peroxidase (10 mg. per ml.) and 25 grams of polyethylene glycol (4000). This composition was made up to a solution having a volume of 250 ml.

A chromogen-containing solution designated solution B was prepared by mixing together 0.70 grams of O-dianisidine dihydrochloride, 12.5 grams of polyethylene glycol (4000), 70 ml. of water. This solution B was made up to a total of 100 ml.

In preparing the article of commerce, 2 ml. of solution A was introduced into a 30 ml. vial and the solution frozen by holding in a refrigerator maintaining a temperature of about plus 10° F. When solution A was a solid, 1 ml. of solution B was introduced into the vial and the contents frozen by again subjecting them to a temperature of about plus 10° F.

The vial was next subjected to freeze drying, i.e., lyophilized to produce substantially water-free solids in the vial.

In adapting the above article of commerce for use, water was added to the vial so that a 30 ml. solution was produced. In order to test for glucose in stabilized whole egg solids, a deproteinized filtrate was prepared as follows:

1.25 grams of whole solids was dissolved in 30 ml. of water. To this was added 10.0 ml. of 0.3 normal barium hydroxide, mixed, then added 10.0 ml. of 5% zinc sulfate, mixed well, then filtered through Whatman No. 12 fluted filter paper.

2 ml. of this clear deproteinized filtrate having a total glucose content of 0.005 mg. was mixed with 1 ml. of the test solution.

After thorough mixing and holding at 30° C. (86° F.) for thirty minutes to allow time for reaction, 10 ml. of 6 normal sulfuric acid was added to the reacting mixture to

arrest the reaction, this amount of sulfuric acid being sufficient to raise the normality of the total mass under test to a normality in excess of 4 normal. At this point, the solution acquired a pink color when measured in a spectrophotometer using light of a wave length of 5400 Angstrom units showing the presence of 0.01% glucose in the original whole egg solids.

EXAMPLE III

A glucose oxidase containing composition, designated solution A, was prepared by mixing together: 200 mg. of glucose oxidase (24,000 units total), 748 ml. of citrate buffer (1 molar solution-pH 5.0), 3.8 ml. of peroxidase (10 mg. per ml.) and 50 grams of polyethyleneglycol (molecular weight of approximately 4000). This composition was made up to a solution having a volume of one liter.

A chromogen-containing solution, designated solution B, was prepared by mixing together: 2.5 grams of ortho dianisidine dihydrochloride, 62.5 grams of polyethyleneglycol (molecular weight of approximately 4000), 250 ml. of water. This solution B was made up to a total of 500 ml. with distilled water.

In preparing the article of commerce, 8 ml. of solution A was introduced into a 125 ml. vial and the solution frozen by holding in a refrigerator maintained at a temperature of about plus 10° F. When solution A was a solid, 4 ml. of solution B was introduced into the vial and the contents frozen by again subjecting them to a temperature of about plus 10° F.

The vial was next subjected to freeze drying, i.e., lyophilized to produce substantially water-free solids in the vial.

In adapting the above article of commerce for use, water was added to the vial so that a 125 ml. of solution was produced.

EXAMPLE IV

A galactose oxidase containing composition, designated solution A, was prepared by mixing together: 8500 mg. of galactose oxidase (24,000 units total), 748 ml. of citrate buffer (1 molar solution-pH 6.4), 3.8 ml. of peroxidase (10 mg. per ml.) and 50 grams of polyethyleneglycol (molecular weight of approximately 4000). This composition was made up to a solution having a volume of one liter.

A chromogen-containing solution, designated solution B, was prepared by mixing together: 2.5 grams of ortho dianisidine dihydrochloride, 62.5 grams of polyethyleneglycol (molecular weight of approximately 4000), 250 ml. of water. This solution B was made up to a total of 500 ml. with distilled water.

In preparing the article of commerce, 2 ml. of solution A was introduced into a 30 ml. vial and the solution frozen by holding in a refrigerator maintaining a temperature of about plus 10° F. When solution A was a solid, 1 ml. of solution B was introduced into the vial and the contents frozen by again subjecting them to a temperature of about plus 10° F.

The vial was next subjected to freeze drying, i.e., lyophilized to produce substantially water-free solids in the vial.

In adapting the above article of commerce for use, water was added to the vial so that a 30 ml. solution was produced. In order to test for galactose in a series of solutions of known galactose concentration, 2 ml. of these solutions having up to a total galactose content of 0.2 mg. were mixed with 1 ml. of the test solution.

After thorough mixing and holding at 30° C. (86° F.) for thirty minutes to allow time for reacting, 10 ml. of 6 normal sulfuric acid was added to the reacting mixture to arrest the reaction, this amount of sulfuric acid being sufficient to raise the normality of the total mass under test to a normality in excess of 4 normal. At this point, the solution acquired a pink color when measured in a spectrophotometer using light of a wave length of 5400

Angstrom units showing the presence of various amounts of galactose. When the absorbencies were plotted versus mg. of galactose on linear graph paper, a straight line resulted from about 0.08 to 0.2 mg. of galactose.

EXAMPLE V

A glucose oxidase containing composition designated solution A was prepared by mixing together: 200 mg. of glucose oxidase (24,000 units total), 748 ml. of citrate buffer (1 molar solution-pH 5.0), 3.8 ml. of peroxidase (10 mg. per ml.). This composition was made up to a solution having a volume of 1 liter.

A chromogen-containing solution, designated solution B, was prepared by heating 22.5 grams of polyethylene glycol (molecular weight of approximately 4000) to 60° C. (140° F.) 500 mg. of ortho dianisidine (free base) was added and the mixture agitated to form a homogeneous solution.

In preparing the article of commerce, 0.23 grams of solution B was poured into a 30 ml. vial and allowed to cool to room temperature, at which temperature the solution set to a solid mass. When solution B was solid, 2 ml. of solution A was introduced into the vial and the contents frozen by subjection to a temperature of about plus 10° F.

The vial was next subjected to freeze drying, i.e., lyophilized to produce substantially water-free solids in the vial.

In adapting the above article of commerce for use, water was added to the vial so that 30 ml. of test solution was produced.

EXAMPLE VI

A glucose oxidase containing composition designated solution A was prepared by mixing together: 200 mg. of glucose oxidase (24,000 units total), 748 ml. of citrate buffer (1 molar solution-pH 5.0), 3.8 ml. of peroxidase (10 mg. per ml.). This composition was made up to a solution having a volume of 1 liter.

A chromogen-containing solution, designated solution B, was prepared by mixing together 250 grams of polyethylene glycol (molecular weight of approximately 6000) and 10 grams of ortho dianisidine (free base) and heated to 65° C. (149° F.). When the dianisidine dissolved in the glycol, the solution was cooled to room temperature. 65 grams of solidified solution was added to 400 ml. of distilled water and when dissolution was complete, the aqueous solution was made up to 500 ml. with additional water.

1 ml. of solution B was added to a 30 ml. vial and the solution frozen by holding in a refrigerator maintained at a temperature of about plus 10° F. When solution B was a solid, 2 ml. of solution A was introduced into the vial and the contents frozen by again subjecting them to a temperature of about plus 10° F.

The vial was next subjected to freeze drying.

In adapting the above article of commerce for use, water was added to the vial so that a 30 ml. test solution was produced.

EXAMPLE VII

A chromogen-containing solution was prepared by heating 22.5 grams of polyethylene glycol (molecular weight of approximately 4000) to 60° C. (140° F.), adding 500 mg. of ortho dianisidine (free base) and agitating until a homogeneous solution was formed.

In preparing the article of commerce, 8 mg. of solution was introduced into a 20 ml. tube and the solution cooled to room temperature at which temperature the solution sets to a solid mass.

A mixture of dry ingredients was prepared containing 63.5 grams of citric acid monohydrate, 132.5 grams of trisodium citrate duohydrate, 38 mg. of peroxidase (RZ 1.0) and 200 mg. of glucose oxidase (24,000 units).

13 mg. of this dry mixture was dispensed into the 20 ml. tube containing the solidified glycol solution.

In adapting the above article of commerce for use, 2 ml. of deproteinized filtrate of desugared whole egg was added to the tube, was mixed into the contents of the tube and incubated at 45° C. for 10 minutes. 10 ml. of 6 normal sulfuric acid was added and the color read against standard. The test indicated the presence of 0.02% glucose on a dry egg powder basis.

EXAMPLE VIII

A chromogen-containing solution was prepared by heating 62.5 grams of polyethylene glycol (molecular weight of approximately 1500) to 55° C. (131° F.), adding 2.5 grams of ortho dianisidine (free base) and agitating until a homogeneous solution was formed. This solution was cooled to room temperature at which temperature the solution hardened into a flowable paste.

To the paste, while it was being agitated in a mixer, was added 63.5 grams of citric acid monohydrate, 132.5 grams of trisodium citrate duohydrate, 38 mg. of peroxidase (RZ 1.0) and 200 mg. of glucose oxidase (24,000 units).

When a uniform dispersion was attained, 525 mg. of the dispersion was introduced into each 30 ml. vial.

In adapting the above article of commerce for use, water was added to the vial so that a 30 ml. test solution was produced. 0.2 ml. of a glucose solution of unknown glucose content containing between about 0.01 and 0.1 mg. of glucose was introduced into a reaction tube. The tube contents were incubated at 37° C. for 10 minutes. 4 ml. of 12 normal sulfuric acid was added and the color read against the standards. The unknown was indicated to contain 0.08 mg. of glucose or 0.4 mg. per ml. or 0.04% glucose.

Although we have described preferred embodiments of the present invention, it will be understood that the description is intended to be illustrative rather than restrictive as details may be modified or changed without departing from the spirit or scope of the invention.

We claim:

1. A method of preparing an article of commerce which upon reconstitution with water may be used for detection of materials that can be reacted with atmospheric oxygen in the presence of oxidase enzymes to produce hydrogen peroxide comprising preparing a water-polyglycol solution of a chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction, said polyglycol being one which is normally solid at 40° C. and substantially soluble in water above about 20° C., lyophilizing the solution of chromogen whereby a substantially water-free solid composition constituted by a minor amount of said chromogen and a major amount of said polyglycol acting as supporting medium, is obtained, preparing oxidase enzyme and buffer salt capable of producing a pH in the range between about 4 and about 7.5 as a separate composition and bringing the compositions together into a single article under conditions maintaining each as distinct entities.

2. A method of preparing an article of commerce which when reconstituted with water may be used for detection of materials reactable with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide comprising preparing an aqueous solution of a polyglycol which is normally solid at 40° C. and substantially soluble in water at temperatures above about 20° C. and an oxidase enzyme specific in its action for said material, said solution being buffered to a pH in the range between about 4 and about 7.5, preparing a separate aqueous solution of a polyglycol which is normally solid at 40° C. and substantially soluble in water above about 20° C. and a chromogen which undergoes color reaction in the presence of reaction products of the enzyme reaction, dehydrating said enzyme and said chromogen solutions to form solid material while maintaining them as substantially separate entities and maintaining the dehydrated

material in solid form until dissolved in water preparatory to use.

3. The method of claim 2 in which the material reactable with atmospheric oxygen is a saccharide.

4. The method of claim 2 in which the material reactable with atmospheric oxygen is a sugar.

5. A method of preparing an article of commerce which when reconstituted with water may be used for detection of sugars comprising preparing an aqueous solution of a polyglycol which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C., an oxidase enzyme specific in its action for a particular sugar, and peroxidase enzyme, said aqueous enzyme solution being buffered to a pH in the range between about 4 and about 7.5, preparing a separate aqueous solution of a chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction and a polyglycol which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C., introducing one of said solutions into a container and solidifying it by freezing, introducing the other of said solutions into said container and solidifying it by freezing, lyophilizing the frozen contents of the container whereby the container contents are maintained as two distinct entities and maintaining the container contents in solid form until dissolved in aqueous medium for use.

6. The method of claim 5 in which the oxidase enzyme is glucose oxidase.

7. A method of preparing an article of commerce which when reconstituted with water may be used for detection of glucose comprising preparing an enzyme solution of glucose oxidase enzyme, peroxidase enzyme, an antioxidant-free polyglycol which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C. and sodium citrate buffer capable of producing a pH in the range between about 4 and about 7.5, preparing a separate aqueous solution of ortho dianisidine dihydrochloride and an antioxidant-free polyethyleneglycol having a molecular weight of about 4000, introducing one of said solutions into a container and solidifying it by freezing, introducing the other of said solutions into said container and solidifying it by freezing, lyophilizing the frozen contents of the container whereby the container contents are maintained as two distinct entities and maintaining the container contents in solid form until dissolved in water preparatory to use.

8. A method of preparing an article of commerce which when dissolved in an aqueous medium may be used for detection of materials reactable with oxygen in the presence of enzymes to produce hydrogen peroxide comprising heating a polyglycol which is normally solid at about 40° C. and substantially soluble in water at a temperature above about 20° C. to a temperature rendering said polyglycol fluid, dissolving chromogen which undergoes a color reaction in the presence of reaction products of an enzyme-oxygen-substrate reaction in said fluid polyglycol, introducing said chromogen solution into a container, cooling said solution to a solidified mass, introducing dry ingredients containing buffer salts and a glucose oxidase enzyme into said container and maintaining the container contents in solid form until dissolved in aqueous medium for use.

9. A method of preparing an article of commerce which when dissolved in an aqueous medium may be used for detection of materials reactable with oxygen in the presence of enzymes to produce hydrogen peroxide comprising heating a polyglycol which is normally solid at about 40° C. and substantially soluble in water at a temperature above about 20° C. to a temperature rendering said polyglycol fluid, dissolving chromogen which undergoes a color reaction in the presence of reaction products of an enzyme-oxygen-substrate reaction in said fluid polyglycol, introducing said chromogen solution into a container, cooling said solution to a solidified mass, preparing an

aqueous solution of an oxidase enzyme specific in its action for said material, said solution being buffered to a pH in the range between about 4 and about 7.5, introducing said enzyme solution into the aforesaid container, solidifying said aqueous solution by freezing, lyophilizing the frozen contents of the container and maintaining the container contents in solid form until dissolved in aqueous medium for use.

10. A method of preparing an article of commerce which when reconstituted with water may be used for the detection of material reactable with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide comprising preparing an aqueous solution comprising about 1 to about 40 units per ml. of oxidase enzyme specific in its action for material reactable with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide, about 7 to about 250 mg. per ml. of buffer salt, about 0.003 to about 0.1 mg. per ml. of peroxidase and about 3.5 to about 100 mg. per ml. of polyethyleneglycol, preparing a separate aqueous solution comprising about 0.1 to about 8 mg. per ml. of chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction and about 2.5 to about 200 mg. per ml. of polyethyleneglycol, which polyglycols are normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C., introducing one of said solutions into a container and solidifying it by freezing, introducing the other of said solutions into said container and solidifying it by freezing, lyophilizing the frozen contents of the container whereby the container contents are maintained as two distinct entities and maintaining the container contents in solid form until dissolved in water preparatory to use.

11. A diagnostic composition comprising an essentially water-free solid material as two discrete unitary masses, one mass comprising a polyglycol solution of an oxidase enzyme specific in its action for a material reactable with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide, and buffering agent capable of producing a pH in the range of between about 4 and about 7.5; and a second mass of essentially water-free material comprising a polyglycol solution of a chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction, said polyglycol being one which is normally solid at about 40° C. and substantially soluble in water above about 20° C., and is present as a major component of the chromogen solution.

12. A diagnostic composition comprising an essentially water-free solid material comprising a lyophilized polyglycol solution of glucose oxidase enzyme, peroxidase and buffer salts adapted to produce a pH in the range between about 4 and about 7.5, as a unitary mass and a lyophilized polyglycol solution of a chromogen which undergoes a color reaction in the presence of enzyme reaction products as a separate unitary mass, said polyglycol being one which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C.

13. An article of commerce comprising a container having as contents, which contents are being maintained in a solid state, a lyophilized polyglycol solution of glucose oxidase enzyme, peroxidase and sodium citrate buffer as a unitary mass and a lyophilized polyglycol solution of ortho dianisidine dihydrochloride as a separate unitary mass, said polyglycol being one which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C. and is present as the major component of the ortho dianisidine dihydrochloride solution.

14. An article of commerce comprising a container having the contents thereof maintained in a solid state as two discrete unitary masses, one mass being a lyophilized polyglycol solution of glucose oxidase enzyme, peroxidase enzyme and buffering agent capable of producing a pH

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in the range between about 4 and about 7.5, and the other mass being a lyophilized polyglycol solution of a chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction, said chromogen and glucose oxidase being present in a ratio of milligrams of chromogen to units of glucose oxidase in the range between about 1 to 10 and 1.5 to 10, said polyglycol being one which is normally solid at about 40° C. and substantially soluble in water at temperatures above about 20° C. and is present as the major component of the chromogen solution.

15. An article of commerce comprising a container having as contents, which contents are being maintained in a solid state, two discrete and essentially water free unitary masses, one mass comprising the product of dehydration of an aqueous solution containing about 1 to about 40 units per ml. of oxidase enzyme specific in its action for material reactable with atmospheric oxygen in the presence of enzymes to produce hydrogen peroxide, 7 to about 250 mg. per ml. of buffer salt, about 0.003 to about 0.1 mg. per ml. of peroxidase and about 3.5 to about 100 mg. per ml. of polyethyleneglycol and a second mass of essentially water-free solid material comprising the product of dehydration of an aqueous solution containing about 0.1 to about 8 mg. per ml. of chromogen which undergoes a color reaction in the presence of reaction products of the enzyme reaction and about 3.5 to about 200 mg. per ml. of polyethyleneglycol, said polyethyleneglycol being normally solid at 40° C. and substantially soluble in water above about 20° C.

16. An article of commerce comprising a container having as contents, which contents are being maintained in a solid state, essentially water-free solid material comprising dehydrated oxidase enzyme specific in its action for substrate material reactable with oxygen in the pres-

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ence of said enzyme and a solidified polyglycol solution of chromogen which undergoes a color reaction in the presence of reaction products of an enzyme-oxygen-substrate reaction, said polyglycol-chromogen solution being present as a separate entity and said polyglycol being one that is normally solid at 40° C. and substantially soluble in water above about 20° C. and is present as the major component of the chromogen solution.

17. An article of commerce comprising a container having as contents, which contents are being maintained in a solid state, essentially water-free solid material comprising the product of lyophilizing a buffered aqueous solution containing oxidase enzyme and a solidified polyglycol solution of chromogen which undergoes a color reaction in the presence of reaction products of an enzyme-oxygen-substrate reaction, said solidified polyglycol solution being present as an entity in direct contact with although separate from said product of lyophilizing a buffered aqueous solution of oxidase enzyme, and said polyglycol being one that is normally solid at 40° C. and substantially soluble in water above about 20° C. and is present as the major component of the chromogen solution.

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ALVIN E. TANENHOLTZ, *Primary Examiner*.