United States Patent [11] 3,607,077

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[21] Appl. No. **820,289**
[22] Filed **Apr. 29, 1969**
[45] Patented **Sept. 21, 1971**

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[32] Priority **May 2, 1968**
[33] **Germany**
[31] **P 17 73 330.1**

[54] COMPOSITIONS OF AGREEABLE ODOR FOR THE DETERMINATION OF ALDOSES

22 Claims, No Drawings

[52] **U.S. Cl.** ..... **23/230 B,** 23/230 R, 252/408
[51] **Int. Cl.** **G01n 21/06**
[50] **Field of Search** 23/230; 252/408

[56] **References Cited**

OTHER REFERENCES

C. A. 67:50965y (1967).

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ABSTRACT: To eliminate the obnoxious odor associated with the *o*-toluidine-glacial acetic method of conducting aldose, especially blood glucose determinations, the color reagent is modified to include at least one hydroxycarboxylic acid, e.g. glycolic acid and little or no glacial acetic acid, there being no sacrifice in the reaction rate or in the yield of the resultant colored compound.

COMPOSITIONS OF AGREEABLE ODOR FOR THE DETERMINATION OF ALDOSES

BACKGROUND OF THE INVENTION

This invention relates to compositions and methods for the determination of aldoses, in particular glucose, in body fluids.

In clinical laboratories, the concentration of aldoses in body fluids must often be determined. Of greatest importance is the quantitative determination of galactose or xylose and, in particular, of glucose, especially blood glucose. Such glucose determinations are used primarily for obtaining a positive diagnosis and then therapy control in diabetes mellitus. In addition, the blood sugar content yields indications for a number of other metabolic disorders (see, in this connection, H. Hallmann, "Klinische Chemie und Mikroskopie" [clinical Chemistry and Microscopy], p. 437, Georg Thieme Publishers, Stuttgart [1950]).

There are many known methods for the determination of aldoses, particularly for the evaluation of blood sugar. Several of the conventional methods, for example the methods known from "Biochem. Zietschrift" [Biochemical Periodical], 135, 46 (1923); "Biochem. Zietschrift", 320, 359 (1950); and "-Munch. med. Wochenschrift" [Munich Medical Weekly], 75, 1301, (1928), are based on detecting the reducing effect of glucose. Since in these so-called reduction methods all reductive substances are simultaneously detected, such analytical methods are unspecific as to glucose in particular.

In another analytical method, glucose is reacted with appropriate enzymes, for example glucose oxidase, and then a determination is made of the products formed by the enzymatic reaction. Although the enzymatic reaction is specific, the indicator reaction is deleteriously sensitive to other chemicals, for example, to reducing agents, such as ascorbic acid. Therefore, the glucose content, for example, in urine, cannot be accurately determined by the glucose oxidase method. In addition, this enzymatic method, just like other enzymatic processes, exhibits the disadvantage that the enzymes have only a limited shelf life.

Still another conventional method for determining glucose is by reaction with aniline, *p*-aminosalicylic acid, *p*-bromoaniline or diphenylamine in glacial acetic acid or also in other acids. This reaction was employed for making individual separate sugars visible on paper- and thin-layer-chromatograms (see, in this connection, for example, J. chrom. 24, 117, (1966) and Scand. J. Clin. Lab. Invest., 20, 216 [1967]. Unfortunately, under the conditions of this determination, there are absorbed aldohexoses, e.g. glucose and galactose, as well as aldopentoses, e.g., xylose, and ketoses, e.g. fructose in a narrow UV-range, so that, for example, the pentoses cannot be measured separately from the hexoses. Consequently, since the reaction is unspecific, this method, like the reduction methods, is not amenable to the determination of unseparated aldose mixtures.

At present, the method generally employed in clinical laboratories for blood sugar determination is described, for example, in Nature 183, 108, (1959), wherein a solution of *o*-toluidine in glacial acetic acid is utilized as the color reagent. This indicator can be stabilized by the addition of thiourea, affecting an autoxidation protection, so that it is readily preservable (Clin. Chim. Acta 7, 140, [1962]). The color formed with glucose is measured, in the *o*-toluidine method, at 620 nanometers, whereas in the aniline/glacial acetic acid method, the measurement must be conducted in the near UV-range. The *o*-toluidine method exhibits the advantage that it is specific for aldohexoses when measuring at 620 nanometers, and that it is essentially specific for aldopentoses when measured at 480 nanometers.

Because of the specificity of the text, the *o*-toluidine method, today, is a standard test in clinical laboratories. However, this method still has a serious practical disadvantage because of the obnoxious odor and irritating effect of the glacial acetic acid used as the solvent. Almost during the entire time of conducting the analysis, i.e., during pipetting the reagent, during the reaction of the glucose at 100° C. in test tubes, during the measuring step in the photometer, and during the cleaning of the containers, the odor is extremely annoying. When conducting several hundred analyses per day, as customary in large clinics, the acetic acid vapor is a most serious problem for the personnel conducting the steps.

To ameliorate, at least partially, the annoyance caused by the odor, it has been suggested to employ 50 percent acetic acid in place of glacial acetic acid ("Arztl. Labor" [Physician's Laboratory] 13, 177–180, [1967]). However, when employing this solvent, the reaction times are longer and the yields of the colored reaction product are substantially diminished, thereby substantially eliminating the practicality of this method for clinical work.

SUMMARY OF THE INVENTION

A principal object of this invention, therefore, is to provide an improved method and reagents for the determination of aldoses, especially glucose, galactose, or xylose.

Upon further study of the specification and claims, other objects and advantages of the present invention will become apparent.

It has been discovered that the above-described disadvantages associated with the determination of aldoses by means of the standard *o*-toluidine method can be avoided by providing that the color reagent contains a hydroxy acid, e.g. mono-, di- and/or tricarboxylic acids substituted by one or more hydroxyl groups. The color reagent may also contain an nonodiferous content of glacial acetic acid and/or an intermediate solvent (Hereinafter referred to as solubilizer).

Accordingly, the invention provides a color reagent for the determination of aldoses, particularly of glucose or galactose or xylose in body fluids, said color reagent comprising:

a. an aromatic amine substituted in the *o*-position and unsubstituted in the *o'*-position, especially *o*-toluidine; and

b. a hydroxy acid, e.g. mono-, di- and/or tricarboxylic acids having hydroxyl groups.

In addition, the color agent can optionally include water, thiourea, glacial acetic acid and/or a solubilizer.

For the determination of aldoses, particularly glucose or galactose or xylose, in body fluids, the color reagent of this invention is added to the sample to be tested, the mixture is heated, and by photometric means, the absorbance of the resultant green colored solution is measured.

A main advantage of the novel color reagent of this invention is that the previously employed obnoxious glacial acetic acid is not required, but if used, it is employed only in a low concentration. Thus, when using the color reagent, there is very little or no irritation caused by the odor. Furthermore, of equal importance is the fact that excellent reaction rates and yields are obtained.

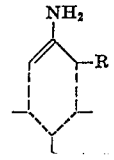
The novel color reagent also permits the test to be adapted to a wide range of conditions, since by adjusting the concentrations of the various components of the color reagent, the rates of color formation and the sensitivity of the test are readily controllable. Accordingly, the color reaction can be made substantially more sensitive, and/or the resultant color more stable than prior conventional processes.

The advantages including the ready formation of the specific color (green with glucose), by the use of the color reagent of this invention are totally unexpected because, for example, halo-fatty acids, thioacids, nonhydroxylated dicarboxylic acids, or inorganic acids are not nearly as effective as the hydroxycarboxylic acids included in the color reagent of the present invention. Besides, organic or inorganic solvents, without the acid addition, cannot be successfully employed for the reaction, either, since no measurable color yields can be obtained therewith. In contradistinction, the color reagent of the present invention, wherein a special group of acids is employed, optionally in combination with a solubilizer, produces remarkably excellent analytical results, with the process being easily adaptable to various test objectives.

DETAILED DISCUSSION OF THE INVENTION

Among the aromatic amines substituted in the *o*-position, but not in the *o'*-position, there are preferred those which are readily distillable.

In general, though, any aromatic amine can be employed which is substituted in the *o*-position but not in the *o'*-position. The general structural formula of the amines preferably used in the reagent according to the present invention is as follows:



wherein the dashed line represents the remainder of an aromatic ring, preferably a carbocyclic ring of 5 or 6 carbon atoms;

R is in particular lower alkyl containing 1–4 carbon atoms, as methyl, ethyl, *n*-propyl, isopropyl or butyl.

The unfulfilled valence bonds, i.e. the positions meta- and para- to the aminocarbon, are satisfied by either hydrogen or any other moiety which does not interfere with the reaction of an aldose, especially glucose, and the aromatic amine to form a colored compound.

For example, the amines can be substituted in the 3- and/or 4- and/or 5-position, preferably by alkyl residues of up to 4 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, or butyl, the *o*-position being also preferably substituted by one of such alkyl residues. The aromatic amines, used in the agents according to the present invention are unsubstituted in the *o'*-position; particularly preferred amines are the *o*-substituted, *o'*-unsubstituted anilines.

Especially preferred are aromatic amines substituted only in the *o*-position, e.g., *o*-toluidine, 2-ethyl-, 2-*n*-propyl-, 2-isopropyl-, 2-*n*-butyl, 2-isobutyl- or 2-tert. butyl-aniline, and also disubstituted amines such as aniline substituted in the 2,3- or 2,4- or 2,5-position by lower alkyl, especially methyl or ethyl, such as 2,3-dimethylaniline, 2,4-dimethylaniline, 2,5-dimethylaniline, 2,3-diethylaniline, 2,4-diethylaniline, 2,5-diethylaniline. These preferred aromatic amines can be readily separated from any interfering autoxidation products, which are always present in larger or smaller proportions, by means of distillation. Especially preferred is *o*-toluidine.

The concentration of the amine has a bearing on the color yield and the stability of the thus-formed dyestuff, so that, by varying the concentration, a reagent can be prepared which is adapted to the specific requirements.

The *o*-substituted and *o'*-unsubstituted amines employed in this invention, result in color reactions, advantageously, only with aldoses. In this connection, aldohexoses form a maximum at 620 nanometers (green color) and can be specifically measured at this wavelength, whereas aldopentoses form a maximum at 480 nanometers (reddish brown coloring).

Though the nature of the aromatic amine is important to the obtaining of the colored compound, such aromatic amines are derived from the prior art. Conversely, the essential novelty of this invention is directed to the use of a hydroxylic acid in conjunction with the amine.

Among such hydroxycarboxylic acids, the preferred group comprises short-chained acids, especially of 2–6 carbon atoms, with 1–5 hydroxy groups and 1–3 carboxy groups and containing only carbon, oxygen and hydrogen atoms.

Hydroxycarboxylic acids of 2–4 carbon atoms, containing 1–2 hydroxy groups and 1–2 carboxy groups are preferred in particular.

Suitable hydroxycarbolic acids include, but are not limited to those which are readily derivable from natural substances, for example hydroxycarboxylic acids derived from sugars, especially hexoses and pentoses, e.g. gluconic acid and mannonic acid. The preferred hydroxycarboxylic acids of this invention are especially the monohydroxymonocarboxylic acids, particularly those of 2–4 carbon atoms, such as glycolic acid and/or lactic acid.

With respect to the dicarboxylic acids useable in this invention, they contain preferably 3–6, especially 4–6, carbon atoms and 1–4 hydroxy groups. Particularly suitable, in this connection, are malic acid and/or tartaric acid. It is also possible to employ the various stereoisomers of these acids, such as *d*-malic acid, l-malic acid, *d*-tartaric acid, l-tartaric acid and/or mesotartaric acid.

Though tricarboxylic acids containing hydroxyl groups, such as citric acid, can also be used, they increase the viscosity of the reagent solution markedly, so that it is preferred in most instances to use hydroxy mono- or dicarboxylic acids. As hydroxytricarboxylic acid citric acid is preferred.

By the use of various hydroxy carboxylic acids there are obtained correspondingly varying reaction rates. Also by varying the concentrations of the hydroxycarboxylic acids, the velocity of the formation of the colored product, as well as the sensitivity of the reagent can be varied within wide limits and can be adapted to the different requirements. Furthermore, of special advantage is the use of particular acid mixtures which prevent crystallization of the reagent even at low temperatures. It is advantageous to utilize such acids which can be readily prepared or purified, especially mixtures of readily crystallizable acids, such as malic and tartaric acid, or mixtures of these readily crystallizable acids with acids that do not crystallize as well, such as lactic acid or glycolic acid. For example, the following mixtures can be employed; malic acid/tartaric acid; malic acid and/or tartaric acid with lactic acid and/or glycolic acid; or also the mixture of glycolic acid/lactic acid.

The concentration in which the total acids are added depends, inter alia, on the solubility of the acids. For example, readily soluble acids, e.g. lactic acid, can be used in the color reagent up to a concentration of 90 percent.

In addition to the hydroxy mono-, di- or tricarboxylic acids, the color reagent of this invention may also contain glacial acetic acid as part of the acid. In general, however, the glacial acetic acid will not be used at all in the color reagent of this invention, or, if so, only in a low concentration, e.g. 10 percent or optionally up to 20 percent, but not more than 30 parts by weight per 100 parts of color reagent in order to avoid annoyance by the odor.

Glacial acetic acid in a low concentration in the reagent may be useful for lowering the viscosity of the mixture and preventing an undesired crystallization of solid components therein contained.

The color reagent of the invention may also contain a solubilizer, especially in the form of organic solvents containing hydroxyl groups and which are miscible with water. Preferred, in this connection, are monohydric alcohols or polyhydric alcohols having a boiling point above 90° C., especially diols. Suitable are, for example, lower alcohols and, in particular, lower diols of up to 4 carbon atoms and having a boiling point of 90° C. or thereabove. Thus, especially effective proved to be diols of 2–3 carbon atoms, such as ethylene glycol, 1,2-propylene glycol or 1,3-propylene glycol, or alcohols of 3–4 carbon atoms, such as *n*-propanol, isopropanol or butanol.

Further preferred solubilizers include monoalkyl glycol ethers, particularly lower glycols etherified in each case with a lower alkyl residue, such as methyl, ethyl, or also butyl glycol ether. The particularly preferred glycol ethers are derived from ethylene or 1,2-propylene glycol, or from 1,3-propylene glycol, such as, for example, ethylene glycol monomethyl (or ethyl or butyl) ether; 1,2-propylene glycol monomethyl (or ethyl or butyl) ether; or 1,3-propylene glycol monomethyl (or ethyl or butyl) ether.

If desired, mixtures of the above-mentioned compounds can likewise be employed as the solubilizer. When employing ethylene glycol monomethyl ether, there is the advantage that the color is formed extraordinarily quickly. On the other hand, unesterified ethylene glycol exhibits the advantage that the

color is extremely stable in the solution. In general, the function of the solubilizer is to lower the viscosity of the reagent, to prevent an undesired crystallization of the solid components therein, to diminish the blank values and to create favorable reaction rates and color yields.

In addition to the solubilizer, consisting generally of hydroxyl-group-containing organic solvents—or in some cases, e.g. when using lactic acid, in place of this solubilizer, water can be employed in the color reagent of this invention. By varying the water content in the reagent, the speed of color formation and the sensitivity of the reagent can be widely adapted to the particular requirements. The use of water is advantageous especially to prevent the formation of inner esters from the hydroxycarboxylic acids, or of organic solvents. Besides, the colored compound formed from the amine and the aldose is somewhat more stable in the presence of water.

By suitably adjusting the proportions of the solubilizer, or of the water, or the hydroxylcarboxylic acids, and/or the glacial acetic acid or the amine, it is possible to obtain the optimum result for the rapid formation of highly stable color.

If desired, other organic solvents can be included in the reagent, as long as they do not interfere with the color formation and the absorbance measurement step. The addition of a small amount of methanol (e.g. up to 15 percent by weight, preferably about 5 percent by weight) is especially valuable for reducing the viscosity of the color reagent. Further suitable solvents which can be added in small amounts are, for example, dioxane, tetrahydrofuran, and glycolic acid esters, e.g. methyl glycolate, such solvents also functioning to lower the viscosity.

In order to improve the stability of the color reagent, the addition of thiourea is recommended.

The following proportions of the above-mentioned individual components of the color reagent of the invention proved to be particularly advantageous: (The data in the following table represent parts by weight per 100 parts by weight of color reagent.)

MIXTURE A	PARTS
Aromatic amine substituted in the *o*-position and optionally in the 3- and/or 4- and/or 5 position, preferably *o*-toluidine	2–25, especially 4–15
Hydroxycarboxylic acids, such as, preferably, glycolic acid, lactic acid, malic acid and/or tartaric acid	5–9.0, especially 15–50
Solubilizers, preferably organic solvents containing hydroxyl groups, having boiling points of 90° C. or higher	0–85, especially 40–80
Water	0–70, especially 0.2–10
Thiourea	0–3, especially 0.02–0.5
Additional inert organic solvents, especially methanol	0–15, especially not more than 5

MIXTURE B

If glacial acetic acid is employed as the additional acid, the proportion of the individual components is changed as follows:

	PARTS
Glacial acetic acid	not more than 30, preferably 10 to 20
Hydroxy, -mono- di- or tricarboxylic acids	3–50, preferably 10–30
Organic solvents containing hydroxyl groups (preferably having a boiling point of 90° C. or higher) as the solubilizers	30–80, preferably 40–60
Aromatic, *o*-substituted amine, water, thiourea and optionally additional inert organic solvent are contained in the same concentration as in mixture A.	

For preparing the color reagent of this invention, the components can be added in any desired sequence. Advantageously, the hydroxyl-group-containing carboxylic acids are dissolved in the solubilizer employed, the latter generally comprising a hydroxyl-group containing organic solvent (having preferably a boiling point of 90° C. or higher), optionally together with water and/or thiourea, and then the aromatic *o*-substituted amine is added thereto. The individual components of the reagent should be employed in the pure form in order to keep the blank values during the measurement low.

The color reagent is utilized for the determination of aldoses, especially of glucose, galactose or xylose, in body fluids, in a conventional manner. Body fluids which can be tested are, in particular, blood, plasma, serum, liquor or urine. In most cases, especially when conducting a blood test, the sample to be examined must first be deproteinized with an agent customarily employed for this purpose, particularly trichloroacetic acid, perchloric acid or uranyl acetate. After the deproteinizing step, the precipitated protein is suitably filtered off or removed by centrifuging before determining the aldose content. When employing serum, plasma or liquor and urine, the deproteinizing step can be omitted, if desired.

For determination purposes, an aliquot of the solution, which was preferably deproteinized, is mixed with a measured quantity of a solution of the agent according to the present invention, and is then heated in a conventional manner for a certain period of time to a fixed temperature for example about 100° C. The periods of time employed range, if the water content is low, between about 4 and 10 minutes, or, if the water content is higher or the temperature is lower, up to about 60 minutes. The exact optimum heating period will be determined in each case for the respective composition of the reagent and the reaction temperature. Suitably, the time is selected so that at the respective reaction temperature, at least 98 percent of the maximum color yield is obtained. In urgent cases, however, it is also possible to operate with briefer periods, since standard and test samples are always treated in the same manner, and since the result reflects only the ratio of the absorbances.

After the mixture has cooled, the absorbance of the solution is measured, at a suitable wavelength, against a blank sample. For example, when using *o*-toluidine, the measurement is preferably conducted in a range of 540–700 nanometers, for example at a maximum of about 620 nanometers. The content of aldose, e.g. glucose, results from a comparison of the determined value with the measured value of a standard sample having a known content. The reaction can be conducted in a conventional manner, for example manually, in test tubes or with the aid of an automatic analyzer, for example analogously to the process described in Clinica Chimica Acta 11, 88 (1965).

For the determination of galactose, any glucose present is previously selectively oxidized by means of glucose oxidase. Then, the galactose content is measured at 620 nanometers in the same manner as in the glucose determination process. When conducting a xylose determination, it is also advantageous to first employ the glucose oxidase oxidation. The xylose content is measured at 480 nanometers in the zone of the absorbance maximum of the reddish brown color.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illus-

trative and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLE 1

The Determination of Glucose

a. Composition of the Color Reagent

10 ml. of *o*-toluidine or 2-ethylaniline or 2-*n*-(or iso-) propylaniline or 2-*n*-(or iso- or tert.) butylaniline
35 ml. of lactic acid (85 percent)
50 ml. of ethylene glycol monomethyl ether
3 ml. of water

b. Conducting a Glucose Determination in the Blood 0.05 ml. of blood and 0.5 ml. of 3 percent trichloroacetic acid are mixed and centrifuged. Subsequently, 0.2 ml. of the thus-obtained protein-free product is mixed with 2 ml. of the reagent described in a, and the solution is heated for 10 minutes in a boiling water bath. After cooling, the absorbance of the solution is measured at 578 nanometers against a blank value. The calculation is conducted by comparing the obtained result with that of a standard sample.

EXAMPLE 2

The Determination of Glucose

a. Composition of Color Reagent

6 ml. of *o*-toluidine or 2,3-dimethylaniline or 2,3-diethylaniline
30 ml. of lactic acid or a mixture of 20 ml. of lactic acid and 10 ml. of citric acid
60 ml. of glycol
13 ml. of water
0.1 g. of thiourea

b. Conducting a Glucose Determination in the Serum 0.02 ml. of serum is mixed with 2 ml. of the reagent described under (a), and heated for 20 minutes to 90° C. After cooling, the absorbance is measured at 623 nanometers against a blank value. The glucose content is read off from a calibration curve obtained in a corresponding manner with solutions having a known glucose content.

EXAMPLE 3

The Determination of Xylose

a. Composition of the Color Reagent

0.6 ml. of *o*-toluidine or 2,4-dimethyl- (or diethyl-) aniline
1.2 g. of malic acid
1.2 g. of glycolic acid
6 ml. of ethylene glycol monomethyl ether
0.5 ml. of methanol
0.5 ml. of water
10 mg. of thiourea

b. Conducting a Xylose Determination (Static Test) 0.1 ml. of urine is mixed with 1 ml. of phosphate buffer (pH = 7.0, 0.1 M) and—for destroying the glucose contained in the sample—with 0.5 mg. of glucose oxidase (30 international units/mg.). The solution is allowed to stand for 30 minutes at room temperature, and then 0.2 ml. of this solution is mixed with 2 ml. of the *o*-toluidine reagent described above under (a). The mixture is heated for 6 minutes in a boiling water bath, or for 30 minutes to 80° C. After cooling, the absorbance is measured at 480 nanometers in a 1 cm. cuvette against a blank value. The content is determined by comparing with the measured value against a standard sample.

EXAMPLE 4

The Determination of Galactose (Static Test)

a. Composition of the Color Reagent

The components set forth in example 3(a) are employed for the reagent.

b. Conducting a Galactose Determination 0.1 ml. of blood is mixed with 1 ml. of a solution of 160 mg. of uranyl acetate and 900 mg. of sodium chloride in 100 ml. of water. The mixture is centrifuged and the thus-obtained protein-free product (0.5 ml.) is mixed with 0.5 ml. of phosphate buffer (pH = 7.5, 0.2 M) and—in order to destroy the glucose—with 0.5 mg. of glucose oxidase. The mixture is allowed to stand for one-half hour. Then, 0.5 ml. of this solution is heated with 5 ml. of reagent solution for 8 minutes to 100° C. After cooling, the absorbance is measured at 620 nanometers against a blank value (2 cm. cuvette). The content is determined by comparison of the measured value with that of a standard sample.

EXAMPLE 5

Glucose Determination

a. Composition of the Color Reagent

0.60 ml. of *o*-toluidine or 2,5-dimethyl-(or -diethyl-)aniline
1 g. of malic acid
1 g. of glycolic acid
6.4 ml. of ethylene glycol monomethyl ether
0.5 ml. of methanol
0.05 ml. of water
0.01 g. of thiourea

b. Conducting a Glucose Determination in Urine

0.2 ml. of a urine specimen from a diabetic, diluted by 1 : 10, is heated with 2 ml. of the reagent described under (a) for 5 minutes to 100° C. After cooling, the absorbance is measured at 632 nanometers against a blank value, and therefrom the glucose content is calculated by comparison with a standard sample.

Analogously, it is also possible to determine the glucose content in plasma, serum or liquor with the aid of the reagent described under (a).

In a modified embodiment, 3.2 ml. of *n*- (or iso-) propanol/3.2 ml. of ethylene glycol monomethyl ether is employed in the reagent defined under (a), instead of 6.4 ml. of ethylene glycol monomethyl ether. The glucose determination by means of the modified reagent is conducted in the same manner as set forth under (b).

EXAMPLE 6

The Determination of Glucose

a. Composition of the Color Reagent

0.6 ml. of *o*-toluidine
1 g. of malic acid
1.5 g. of lactic acid (80 percent)
6 ml. of ethylene glycol monomethyl ether
10 mg. of thiourea

b. Conducting Glucose Determination in Whole Blood

0.1 ml. of the specimen is mixed with 1 ml. of a solution of 5 g. of trichloroacetic acid in 100 ml. of water. The mixture is centrifuged and 0.2 ml. of the thus-obtained protein-free product is mixed with 2 ml. of the reagent described under (a); then, the mixture is heated for 6–8 minutes in a boiling water bath. After cooling, the absorbance is measured at 578 nanometers against a blank value. The calculation is conducted with the aid of a calibration curve, or by comparison with the measured values of a standard sample.

EXAMPLE 7

The Determination of Glucose, Galactose or Xylose

Composition of the Reagent

1 ml. of *o*-toluidine
2 ml. of glacial acetic acid
1 g. of malic or tartaric acid
5 ml. of ethylene glycol monoethyl ether
1 ml. of water
10 mg. of thiourea

The determination is conducted analogously to example 4.

In a variation of the above-described composition, the following are alternatively employed in place of 5 ml. of ethylene glycol monoethyl ether:

a. a mixture of 4 ml. of ethylene glycol monoethyl ether and 1 ml. of 1,2-propylene glycol monomethyl (or ethyl) ether; or

b. a mixture of 3 ml. of ethylene glycol monomethyl ether and 2 ml. of 1,3-propylene glycol monomethyl (or ethyl) ether; or

c. a mixture of 4.5 ml. of ethylene glycol monomethyl ether and 0.5 ml. of butanol.

The determination of aldoses by these reagents are likewise conducted analogously to example 4.

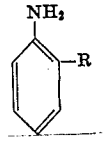
The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed is:

1. A color reagent suitable for the determination of aldoses, said color reagent comprising:

a. an aromatic amine of the formula



wherein R is lower alkyl, and the hydrogen on the carbons meta- and para- to the NH_2 —substituted carbon may be substituted by a moiety which is noninterferring to the aldose-aromatic amine color reaction;

b. a hydroxy acid selected from the group consisting of hydroxy mono- di- or tricarboxylic acids and mixtures thereof, and

c. 0–30 parts by weight of glacial acetic acid per 100 parts by weight of color reagent.

2. A color reagent as defined by claim 1 wherein said hydroxy acid comprises a lower hydroxymonocarboxylic acid.

3. A color reagent as defined by claim 1, wherein said hydroxy acid comprises a member selected from the group consisting of glycolic acid, lactic acid, malic acid, tartaric acid and citric acid.

4. A color reagent as defined by claim 1 further comprising an inert hydroxy containing organic solvent having a boiling point of at least 90° C.

5. A color reagent as defined by claim 1 wherein, based on 100 parts by weight of total color reagent, said *o*-substituted, *o'*-unsubstituted aniline comprises 2–25 parts and said hydroxy acid comprises 5–90 parts.

6. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 1.

7. A color reagent as defined by claim 1 wherein the said meta- and para-positions are satisfied by hydrogen or alkyl of 1–4 carbon atoms.

8. A color reagent as defined by claim 1 wherein the said meta- and para positions are satisfied by hydrogen.

9. A color reagent as defined by claim 1 comprising 10–20 parts by weight of glacial acetic acid.

10. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 9.

11. A color reagent as defined by claim 1, containing zero parts by weight of glacial acetic acid.

12. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 11.

13. A color reagent as defined by claim 1 wherein said *o*-substituted, *o'*-unsubstituted aniline is *o*-toluidine.

14. A color reagent as defined by claim 13 wherein said hydroxy acid comprises a lower hydroxymonocarboxylic acid.

15. A color reagent as defined by claim 13 wherein said hydroxy acid comprises a mixture selected from the group consisting of (1) malic and lactic acid, (2) malic and glycolic acids, (3) tartaric and lactic acids, and (4) tartaric and glycolic acids.

16. A color reagent as defined by claim 13 further comprising a solubilizer selected from the group consisting of a glycol and a monoalkyl glycol ether.

17. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 13.

18. A color reagent as defined by claim 13 wherein said hydroxy acid comprises a member selected from the group consisting of glycolic acid, lactic acid, malic acid, tartaric acid and citric acid.

19. A color reagent as defined by claim 18 further comprising a solubilizer selected from the group consisting of ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, ethylene glycol, propylene glycol and mixtures thereof.

20. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 18.

21. A color reagent as defined by claim 18 wherein, based on 100 parts by weight of total color reagent, said *o*-substituted, *O'*-unsubstituted aniline comprises 2–25 parts and said hydroxy acid comprises 5–90 parts.

22. In a method of conducting an aldose determination comprising the steps of mixing a color reagent with a liquid containing an aldose, and photometrically measuring the resultant color,

the improvement comprising employing a color reagent as defined by claim 21.