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PROCESS FOR DETERMINING SUGAR CONTENT IN BLOOD

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2 Claims. (Cl. 252—408)

The present invention relates to the manufacturing of reagent in powder form by intimately mixing reducible heavy metal salt, acid, alkali hydroxide and alkali carbonate in dried solid state. They are used for sugar determination without the use of external heating.

Various kinds of reagents so far invented for the determination of reducing sugars are unsatisfactory in that they are either unstable or not sensitive enough to determine blood sugar concentration which amounts to a few tenths of a percent or less. The reason for this is that highly hygroscopic substances are employed or the heat evolved in the reaction is not sufficient to cause the reduction of heavy metal salt to a satisfactory degree since it utilizes merely the heat of solution.

The present inventor has observed that a mixture of a non-hygroscopic and non-reducing, soild acid, for instance alkali dihydrogen phosphate, alkali bisulfate, phthalic acid, maleic acid, succinic acid etc. or their anhydrides, and alkali hydroxide is sufficiently stable and on the addition of a few drops of water the mixture produces sufficient amount of heat to reduce a reducing heavy metal salt in the presence of a reducing sugar.

If to a mixture of the composition indicated in Example 1 one to three drops of water containing a reducing sugar like glucose, lactose, etc. is added, grey to black shade develops, which is proportional to the sugar concentration due to the reduction of the heavy metal salt.

Such a mixture gives positive reaction to a test solution containing a reducing sugar above 0.01 percent, although the penetration of the test solution is not so rapid that rather vigorous shaking of the mixture is required to render the reduction to take place within one minute.

When anhydrous alkali carbonate, anhydrous sodium carbonate or lithium carbonate is added as indicated in Example 2 to the reagent indicated in Example 1, the penetration of the test solution into the powder-reagent takes place within thirty seconds.

Example 1

	Percent
Dried sodium hydroxide	52
Potassium acid phosphate	41
Bismuth oxychloride	7

Example 2

	Percent
Dried sodium hydroxide	40
Potassium acid phosphate	48
Bismuth oxychloride	6
Anhydrous sodium carbonate	6

In Example 1 or 2, sodium hydroxide may be replaced by potassium hydroxide or other alkali or alkaline-earth hydroxide, potassium acid phosphate by potassium bisulfate or by other non-hygroscopic solid acid, bismuth oxychloride by bismuth oxide or by any other non-hygroscopic reducible heavy metal salt, and anhydrous sodium carbonate by anhydrous lithium carbonate, etc.

Each ingredient of Examples 1 and 2 is separately dried and mixed intimately.

Determination of reducing sugar in solution

Take approximately two tenths gram of the powder-reagent in a small test-tube and add to it two to three drops of test solution, for instance, urine, beverage, etc. and shake the tube. In one half to one minute there is produced a color which depends upon the heavy metal salt employed. With bismuth salt, grey to black shade appears, which is proportional to the sugar concentration which may be estimated by comparing the shade developed with that of the standard previously prepared.

The reagent with bismuth salt as a reducing heavy metal turns yellowish by a solution containing no reducing sugar, faint grey by that containing 0.02 percent reducing sugar, and black by that containing 1.0 percent. For the determination of the sugar at higher concentration than 1.0 percent ordinary dilution method may be used.

The advantage of the present reagent over other similar reagents is that its reaction is not interfered with urine albumen or uric acid or with medicaments such as hexamethylenetetramine, acetylsalicylic acid, etc. This is due to the fact that with this reagent the reduction by reducing sugar takes place almost instantly and the reaction mixture cools off to solidify before such interfering substances decompose to reducing intermediates.

Determination of blood sugar

Protein of both blood plasma and serum gives slightly orange color, thus interfering with the color comparison. Blood sugar determination should, therefore, be performed after the protein has been removed. For this purpose add three drops of the protein precipitation-reagent indicated below together with thirteen drops of water to four drops of either plasma or serum. White protein precipitate produced is then filtered off or centrifuged off. Two drops of the filtrate is now added to the sugar reagent and the color developed is compared with standard to determine the sugar concentration of the filtrate. The percentage of sugar thus obtained is multiplied by five to obtain percentage of sugar in the blood. By this method the filtrate containing 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 percent sugar may be differentiated within five minutes.

Reagent for protein precipitation

Sodium xylenesulfonate	33 gm.
Potassium bisulfate	30 gm.
Brom phenol blue	Sufficient amount to color the solution.
Water	Up to 100 cc.

The sodium xylenesulfonate may be replaced by any aromatic sulfonate without so-called positive radical like amino group.

Brom phenol blue is used to indicate the complete precipitation of the protein when it is yellow. It has been found that the protein precipitation is incomplete at a pH higher than 3 beyond which the indicator turns green to blue violet.

I claim:

1. A process for determining sugar content in blood which comprises first precipitating protein in the blood at a pH below 3 by treating the blood with a protein precipitation reagent consisting essentially of a sodium aryl sulfonate, alkali metal bisulfate, an indicator and water, and adding to the protein-free blood, a dry reagent consisting essentially of a dry intimate mixture of from 6 to 7% of a reduceable bismuth salt, from 48 to 41% of a non-hygroscopic, non-reducing solid acid and

from 40 to 52% of an alkali hydroxide, said solid acid and alkali hydroxide being present in amounts sufficient to generate enough heat, when said reagent is moistened with said blood, to reduce said bismuth salt, and provide a quantitative indication of the sugar content in said blood.

2. The process of claim 1 wherein said protetin precipitation reagent consists of 33 grams sodium xylenesulfonate, 30 grams potassium bisulfate, Brom phenol blue and water up to 100 cc.

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