COMPOSITION FOR DETECTING KETONE BODIES AND METHOD OF PREPARATION

BACKGROUND OF THE INVENTION

This invention relates generally to a composition and method for detecting ketone bodies in human fluid and, more particularly, to an improved composition, test means, method of making a test means and process for the detection of ketone bodies in human fluids which is accurate over a wide range of acidity and alkalinity.

It is known that ketosis takes place owing to insufficient intake to sugars or other disorders of sugar availability in living bodies. In patients with ketosis, ketone bodies such as acetoacetic acid, acetone or beta hydroxybutyric acid (considered to be a ketone body) increase in the blood and they are excreted primarily as acetoacetic acid in the urine. Such ketosis indicates probable presence of one or more diseases such as diabetes, disorders of the digestive organs, renal insufficiency, uremia, malignant carcinoma, etc. Thus, detection of ketone bodies in body fluids such as urine, serum or plasma is important to provide early diagnosis of such diseases.

The use of chromogens such as soluble nitroprussides in the detection of ketone bodies has long been recognized. For example, as early as 1940, U.S. Pat. No. 2,186,902, to Fortune, disclosed a formulation where nitroprusside is reacted in the presence of ammonia to develop particular colorations. In 1950, U.S. Pat. No. 2,509,140 to Free disclosed formulations for the detection of ketone bodies in urine where the formula contained the combination of water soluble nitroprusside, an aliphatic amino acid (glycine) and an alkaline material. Shortly thereafter, in 1951, U.S. Pat. No. 2,577,978 to Nicholls disclosed the addition of lactose or similar sugars to the formulation described in the Free patent to enhance the utility of the test and the reliability of results.

In 1961, U.S. Pat. No. 2,990,253 to Smeby described a test composition incorporated into a bibulous strip or stick. Since it is known that nitroprusside is unstable in an alkaline aqueous medium, the nitroprusside was kept separate using a two-step preparation method where the nitroprusside was first applied to the bibulous carrier in an acid aqueous medium, thus preserving the stability of the nitroprusside and, after the nitroprusside was dried, the carrier was dipped into a non-aqueous solution of organic bases such as amines or amino alcohols to achieve the necessary alkalinity for the bibulous strip. Such alkalinity was necessary because the nitroprusside per se functioned properly only at alkaline pH even though unstable at alkaline pH. The reference to the nitroprusside being unstable in an alkaline aqueous medium means that in such a solution the nitroprusside will react with certain negative ions including not only acetoacetate ions but even with hydroxy ions thus providing an unacceptable result by the formation of a yellow colored hydroxy ferricyanide. The reference to nitroprusside per se functioning properly only at alkaline pH means that as of the 1961 state of the art, the ketone ionized as a negative ion, to thus react with the nitroprusside, only in alkaline solutions.

There were, however, problems with the technique described in the Smeby patent. For example, the amines and amino alcohols are hygroscopic and volatile. Because the amines and amino acids are hygroscopic, when they absorb moisture from the air, the moisture provided the hydroxy ion and since the strip was alkaline this resulted in the aforementioned instability of the nitroprusside, e.g., this permitted the formation of hydroxy ferricyanide.

In 1965, U.S. Pat. No. 3,212,855 to Mast disclosed an improvement in the two-dip method of preparing a test strip or bibulous strip where the strip was first impregnated with an alkaline buffer and an amino acid combination. After drying, the strip was then impregnated with the combination of an alkali metal nitroprusside, an organic film-forming compound of acid pH and an organic solvent. The test strip prepared according to the description in the Mast patent resulted, however, in two problems. First, the organic solvent, dimethyl sulfoxide, is a health hazard and, second, the test strip had insufficient humidity stability to function properly. Thus, upon absorption of moisture, the nitroprusside hydrated, became alkaline and unstable.

Then, in 1975, U.S. Pat. No. 3,880,590 to Ogawa described a test strip made from a single dip or impregnation of a bibulous carrier into the combination of nitroprusside and a heavy metal salt such as nickel chloride. Again, a hazardous solvent, dimethyl sulfoxide, was used. The heavy metal salt provided a positive metal ion and shifted the ionization constant of the ketone so that the ketone ion was negatively charged in an acid pH solution. Hence the nitroprusside would theoretically function properly in an acid pH. If the body fluid such as urine was expected to be acidic, a buffer such as TRIS was added to the solution prior to dipping the bibulous carrier into the solution. There were, however, several problems and disadvantages with the system described in the Ogawa patent. The solution itself was unstable, there was low sensitivity to the ketone bodies and there was relatively no humidity stability. Lack of humidity stability is a serious shortcoming because of the hygroscopic nature of both the TRIS and the nitroprusside.

More recently, U.S. Pat. No. 4,147,514 issued to Magers and Tabb in 1979 disclosed an improved solution using nitroprusside in combination with at least one inorganic metal salt with the metal selected from the group of magnesium and calcium and, optionally, at least one primary amine. This is a one-dip application for impregnating the chemicals onto a bibulous carrier and exhibits humidity stability. However, the solution itself demonstrates some instability and a very low buffer capacity in that body fluids of a very high alkalinity or high acidity will give false readings.

Thus is may be seen, from a review of the above, that each of the prior art techniques yielded at least one significant drawback.

The present invention overcomes the shortcomings of the prior art by providing a new and improved test means, method of making the test means and method of testing for ketone bodies which is operable over a wide range of pH, where the solutions themselves are stable, where the test strip exhibits high humidity stability and where the system has a high degree of sensitivity to the ketones thus providing accurate, reliable results.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The preferred embodiment of the present invention is a test strip of a bibulous matrix which is insoluble in and maintains its structural integrity when exposed to water or physiological fluids. The preferred method for the preparation of such a test strip is a two-dip application