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URIC ACID TEST PROCEDURE

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ABSTRACT OF THE DISCLOSURE

Test procedure which is useful in the determination of uric acid in body fluids. The test reagent comprises a solution of 2,4-dimethylquinoline in ethyl alcohol in the presence of sulfuric acid. Test reagent turns brown in the presence of uric acid and can be used for the qualitative and quantitative determination of uric acid.

BACKGROUND OF THE INVENTION

Uric acid is normally present in the body fluids of warm blooded animals. For diagnostic purposes it is often desirable to ascertain the uric acid level of certain body fluids. In accordance with this invention the uric acid content of body fluids can be readily and inexpensively determined.

The metabolism of warm blooded animals produces uric acid in varying amounts from ingested foods. In a given warm blooded animal the uric acid content of a body fluid must be held within carefully prescribed limits in order to prevent undesirable consequences.

For example, in a human being if the uric acid content of blood serum exceeds certain limits for a given individual uric acid tends to precipitate out in crystalline form in the body joints. These crystals of the uric acid tend to inflame the joint in such a fashion that the joint in question becomes very painful. This malady is commonly referred to as "gout".

Due to the fact that the abnormal presence of uric acid can cause severe problems, it is desirable to have a quick and easy test for the determination of uric acid in body fluids. The prior art includes quantitative and qualitative tests for the determination of uric acid in body fluids. However, most prior art test procedures depend on the fact that the enzyme uricase quantitatively and qualitatively degrades uric acid. Hence, these prior art testing procedures are enzymatic in nature. Because these prior art testing procedures are enzymatic the variables in the test must be carefully controlled and they require a definite incubation period in which to allow the enzyme uricase to function.

In contrast with this prior art, the test procedure of this invention is not enzymatic and gives a direct reading. The general test procedure of this invention is colorimetric in nature and hence can be used as a qualitative and quantitative indicator for the presence of uric acid. While many of the prior art test procedures, due to the fact that they are enzymatic, require a definite incubation period, the test procedure of this invention does not require an incubation period and gives a reading immediately. Likewise, due to the fact that the test procedure of this invention is quick and relatively simple, it can be performed directly in the doctors' office without the need for taking the patent's sample, sending the sample out for analysis and waiting for an extended period of time for the results of the analysis. Accordingly, in an emergency situation, the fact that the test procedure of this invention can be performed quickly can be very significant.

SUMMARY OF THE INVENTION

This invention is concerned with a test procedure for uric acid in body fluids. The test procedure of this invention comprises adding to a sample of a body fluid a solution of 2,4-dimethylquinoline in ethyl alcohol. After this addition the pH of the solution is lowered by the addition of sulfuric acid. Upon the addition of sulfuric acid a brown color develops in the presence of uric acid. This brown color absorbs strongly in the region of 532μ, and accordingly can be used as a qualitative and quantitative indicator for the presence of uric acid.

The primary object of this invention is a superior test procedure for the presence of uric acid in body fluids.

Another object of this inventon is a non-enzymatic test procedure for uric acid in body fluids.

Still another object of this invention is a test procedure for uric acid which will give an instantaneous reading.

Finally the objects of this invention include all the other novel features which will be obvious from the specification and claims at hand.

DESCRIPTION OF THE PREFERRED EMBODIMENT

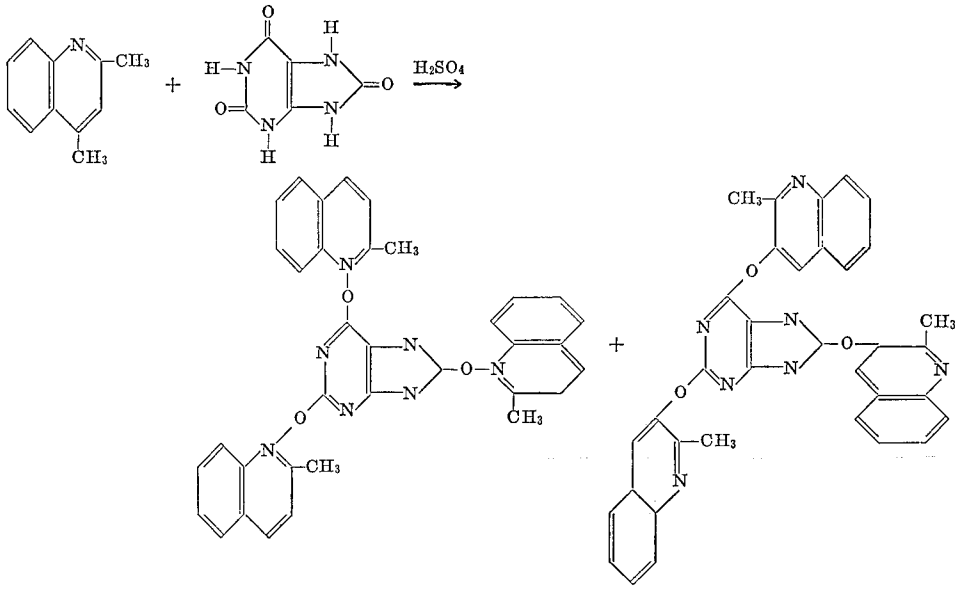
The subject invention relates to a process wherein a solution of 2,4-dimethylquinoline in ethyl alcohol is used as a colorimetric indicator for uric acid in the presence of sulfuric acid. When the solution of 2,4-dimethylquinoline is added to a body fluid containing uric acid and sulfuric acid is added thereto the resulting solution turns brown.

For use in accordance with this invention, the concentration of 2,4-dimethylquinoline in ethyl alcohol can be from about 1% to about 10% by volume. A more preferred range for this concentration is from about 1% to about 5%, while a most preferred concentration is 2%.

When the solution of 2,4-dimethylquinoline in ethyl alcohol is added to a body fluid and the pH of the solution is lowered with sulfuric acid, a brown complex is formed in accordance with Equation 1 below. It is to be noted that while applicant is not sure of the exact mechanism whereby the brown complex is produced, it is

thought that Equation 1 accurately represents the process of this invention.

Equation 1



For purposes of developing the brown complex a stoichiometric amount of 2,4-dimethylquinoline in ethyl alcohol is needed as based on the amount of uric acid present in the body fluid sample. While a stoichiometric amount of the test reagent is all that is needed in accordance with this invention, it is desirable to utilize an excess of the test reagent. Generally, it is preferred that an excess on the order of 30 to 40 percent over the stoichiometric amount be used for purposes of developing the brown color in accordance with this invention.

After the solution of 2,4-dimethylquinoline in ethyl alcohol is added to the body fluid sample, the pH of the composite solution is lowered to the range of from about .1 to 3 by the addition of concentrated sulfuric acid. For use in accordance with this invention, concentrated sulfuric acid is added until the concentration of sulfuric acid in the overall solution is on the order of 30 to 50 percent. A more preferred range for this concentration is from about 45 to about 50 percent, while a most preferred concentration for use in accordance with this invention is 49 percent.

It is to be noted that for use in accordance with this invention the pH of the composite solution must be lowered with sulfuric acid. That is, the color reaction of this invention is not strictly a function of pH, but instead it is thought that the sulfate ions produced by the sulfuric acid have a part in the reaction and aid in the formation of the brown complex of this invention. Due to this fact, sulfuric acid must be utilized as a pH lowering medium.

The test procedure of ths invention can be carried out at any convenient temperature. However, it is preferred that the test of this invention be carried out at room temperature.

The subject test can be carried out on many types of body fluids. For example, as a starting material the test of this invention can utilize blood serum, plasma, urine, etc. Likewise, the invention is applicable to standard uric acid solutions such as solutions of uric acid in a buffered acidic medium.

It is within the purview of this invention to add to the compositions of this invention compatible materials which do not affect the basic and novel characteristics of the composition of this invention. Among such materials are coloring agents, including dyes and pigments, and similar additives. Additives such as antioxidants, stabilizers and antifoaming, may also be aded. The upper limit of the

quantity of additives is usually about 2 weight percent of the product.

The following examples will illustrate the subject invention. These examples are given for the purpose of illustration and not for purposes of limiting this invention. (All parts percent are given by weight unless otherwise specified.)

EXAMPLE I

A reagent was prepared by adding 1 ml. of 2,4-dimethylquinoline to 100 ml. of 95% ethanol (Formula No. 3). The resulting solution was continuously stirred until the 2,4-dimethylquinoline was completely dissolved. Whereupon the solution was covered to prevent evaporation and protected from light.

To a small vial was added .25 ml. of the above described reagent. Likewise .01 ml. of blood serum was added. To the resulting solution was added 0.25 ml. of 98% concentrated sulfuric acid. The resulting solution was mixed and allowed to cool slightly.

Upon the addition of the concentrated sulfuric acid the solution turned dark brown indicating the presence of uric acid in the blood serum. A reading was taken at 532μ on a Coleman Colorimeter Model 124 as produced by the Coleman Instrument Co. This reading indicated that the concentration of uric acid in the blood serum was 4.5 gram percent. This is to be compared with the concentration of the serum sample which was 4.6 gram percent.

EXAMPLE II

Using the reagent and test procedure of Example I, the test was repeated using hydrochloric acid in lieu of sulfuric acid. Upon the addition of the hydrochloric acid an immediate precipitate was formed and there was no colorimetric reading in the range of 532μ. Due to the fact that an immediate precipitate was formed, concentrated hydrochloric acid is not an acceptable substitute for the sulfuric acid of this invention.

EXAMPLE III

Using the reagent and test procedure of Example I concentrated nitric acid was substituted for the concentrated sulfuric acid. Upon the addition of the concentrated nitric acid, nitrous oxide was formed and was emitted from the solution in the form of a brown gas. The resulting solution did not absorb in the region of 532μ. Due to the fact that the solution did not absorb in this range and the fact that nitrous oxide was formed,

this test procedure could not be utilized as an analytical tool for the determination of uric acid.

EXAMPLE IV

Using the reagent and test procedure of Example I concentrated acetic acid was substituted for the concentrated sulfuric acid of Example I. No reaction occurred and the solution did not absorb in the range of 532μ. Because it did not absorb in this range, concentrated acetic acid cannot be substituted for the concentrated sulfuric acid of this invention.

EXAMPLE V

Using the reagent and test procedure of Example I the uric acid content of a human serum sample was determined by the absorption in the range of 532μ which indicated that the sample had a uric acid content of 4.8 gram percent. This is to be compared with the known concentration of the serum sample in question which was 4.9 gram percent.

EXAMPLE VI

Using the reagent and test procedure of Example I uric acid content of a serum control was determined. The absorption in the range of 532μ was indicative of the fact that the uric acid content of the serum control was 10.3 gram percent. This is to be compared with the known uric acid content of the sample which was 10.5 gram percent.

EXAMPLE VII

A standard uric acid solution was prepared by dissolving 10 grams of uric acid (C.P. grade) in 70 ml. of distilled water whereupon 5 ml. of concentrated hydrochloric acid was added and the solution was diluted to 100 ml. with distilled water. Using the reagent and test procedure of Example I, it was determined that the percent transmissions of various concentrations of the standard solution are in accordance with Table I. From Table I, it can be seen that the percent transmission is a function of the uric acid concentration. With the data of Table I a standard curve can be set up which can be used in unknown determinations.

TABLE I

Uric acid concentration in gram percent:	Percent transmission
10	43
8	62
6	75
5	80
4	84
0	98

What is claimed is:

1. A test reagent for uric acid in body fluids comprising sulfuric acid and a solution of 2,4-dimethylquinoline in ethyl alcohol.

2. The test reagent of claim 1 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is from about 1 to about 10 percent by volume.

3. The test reagent of claim 1 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is from about 1 to about 5 percent by volume.

4. The test reagent of claim 1 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is 2 percent by volume.

5. A testing procedure for the determination of uric acid in a body fluid which comprises the steps of bringing said body fluid into contact with a solution of 2,4-dimethylquinoline in ethyl alcohol and adding thereto sulfuric acid.

6. The process of claim 5 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is from about 1 to about 10 percent by volume, and the concentration of sulfuric acid in the overall test solution is from about 30 to about 50 percent.

7. The process of claim 5 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is from about 1 to about 5 percent by volume, and the concentration of sulfuric acid in the overall test solution is from about 45 to about 50 percent.

8. The process of claim 6 wherein the concentration of 2,4-dimethylquinoline in ethyl alcohol is 2 percent by volume and wherein the concentration of sulfuric acid in the overall test solution is 49 percent.

9. The process of claim 6 wherein a stoichiometric excess of from about 30 to about 40 percent of 2,4-dimethylquinoline in ethyl alcohol solution is used as based on the stoichiometric relationship of the reaction of 2,4-dimethylquinoline with uric acid.

10. The process of claim 6 wherein a .01 ml. portion of a body fluid is utilized in conjunction with .25 ml. of a solution of a 1 percent by volume of 2,4-dimethylquinoline in ethyl alcohol and .25 ml. of 98 percent concentration sulfuric acid which is added thereto, wherein the qualitative and quantitative uric acid content of said body fluid is determined by colorimetric determination at about 532μ.

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