United States Patent Office 3,536,448
Patented Oct. 27, 1970

3,536,448

URIC ACID DETECTION

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No Drawing. Filed July 28, 1967, Ser. No. 656,652
Int. Cl. G01n 21/20, 21/22, 33/16
U.S. Cl. 23—230 11 Claims

ABSTRACT OF THE DISCLOSURE

An alkaline phosphotungstate process and a composition for detecting uric acid in which a composition comprising a color enhancer and an amino carboxylic sequestering agent is utilized in combination with a phosphotungstate reagent.

Background of the invention

This invention relates to the detection of uric acid and more particularly to a new process and novel composition for the detection of uric acid in fluids.

Uric acid is normally present in some quantities in the fluids of mammals, particularly humans, as a result of normal body functions. Concentrations of uric acid, especially in the blood and urine, provide easily obtainable and correlatable indications of body conditions which may be adverse to general good health.

For example, ingestion of foods, such as purine-containing foods, normally has no effect on the uric acid blood level. An unexplainable evaluation of such concentration may, however, be indicative of renal insufficiency. In some pathological conditions not related to dietary ingestion, such as uremia and gout, an abnormal increase occurs in the concentration of uric acid in the blood. Unusual levels of uric acid in the blood are also known to be associated with conditions that cause excessive destruction of the nuclei of white blood corpuscles, for example, lukemia and pneumonia.

Medical science has long recognized the usefulness of a test for uric acid as an aid in diagnosing the foregoing conditions, and in some instances, distinguishing between closely related abnormal conditions, for example, gout and arthritis. Gout is characterized by an abnormal increase in uric acid in the blood whereas arthritis does not exhibit such increase. For such reasons it has been considered desirable to have a test which is simple and inexpensive and yet provides a precise determination of uric acid concentration.

Uric acid is generally found in the blood in quantities varying between about 0.7 and 7.0 milligrams per 100 milliliters, reported as mg. percent. This percent varies over a wide range as a result of exercise, diet and similar factors. With abnormal conditions, values of blood uric acid concentrations of the blood in excess of two and three times their usual value often occur.

Two major processes have been developed for detecting uric acid and are broadly described as the uricase method and the alkaline phosphotungstate method.

The uricase method is based on the principle that uric acid has an ultraviolet absorption peak in the range of 290 to 293 mμ, whereas the end products of the reaction of uricase and uric acid have no absorption at this wavelength. To determine uric acid, serum is reacted with a uricase enzyme and the decrease in absorbance is measured. This method, while providing accurate determinations, requires highly skilled laboratory personnel, expensive equipment and a great deal of time to perform.

Alkaline phosphotungstate determination of uric acid was developed about 1894 and has been subjected to many alterations since that time. For detection of uric acid in blood with this procedure, phosphotungstic acid is added to a protein free filtrate derived from serum. Many alkalizing agents, such as sodium carbonate, sodium cyanide, sodium glycerosilicate and sodium tungstate, have been used in varying processes to increase the reaction or indication speed, to increase sensitivity, to reduce turbidity or cloudiness during the reaction, or to adjust other conditions affecting the quality of the test.

The alkaline phosphotungstate process, because of its lack of sensitivity and adverse side reactions that caused conditions such as turbidity, often required an excessively large serum sample. Also, the time required for completion of the color change indicating uric acid concentration was excessive, generally being in excess of 60 minutes.

Summary of the invention

Therefore, it is an object of this invention to provide a process that requires but a short time for completion of an indicating color change in the detection of uric acid.

A further object of this invention is to provide a process that is free of the adverse side effects usually associated with previously known processes for the detection of uric acid.

Another object of this invention is to provide a process of increased sensitivity for detection of uric acid.

An additional object of this invention is to provide an improved composition of greater sensitivity and reliability for detecting uric acid.

This invention is embodied in an improved process for detecting uric acid in a fluid containing the same, in which process a phosphotungstate reagent is reacted with the uric acid in the fluid to yield a color change, the improvement comprising mixing with the fluid a color enhancer and a sequestering agent prior to reacting the phosphotungstate reagent with the uric acid present.

The invention is also embodied in a composition for use in detecting uric acid, the composition including a color enhancer, a sequestering agent and a phosphotungstate reagent.

In this application the term "detection" includes the determination of the presence as well as of the concentration of uric acid in a fluid.

Description of the preferred embodiments

Uric acid may be readily and accurately detected in a fluid according to the novel process and with the novel composition of this invention. The reliability of the results of this detection is partially dependent upon the proper preparation of the fluid to be tested. A commonly tested fluid for uric acid is blood which is considered a complex fluid because it includes many interfering components. To obtain accurate results these interfering components are treated in such a way that they do not interfere with the uric acid indication. The process and composition of the invention will be described as they are utilized for detection of uric acid in blood. However, it is understood this process and composition are not limited to detection of uric acid in blood and may be used with other fluids.

In preparing a sample of blood for detection of uric acid, the whole blood is allowed to separate by natural coagulation into layers of blood cells and serum. The serum is drawn off and retained for further treatment. Only a small amount of serum, between about 0.3 ml. and 0.5 ml., is required for detection of uric acid by the practice of this invention as a result of the increased sensitivity and freedom from extraneous interference which characterizes the invention.

The serum is preferably further treated to remove pro-

tein. This may be done according to the known chemical procedure in which sulphuric acid and sodium tungstate are mixed with the serum, forming a precipitate that removes the protein. The supernatant and precipitate may be separated by centrifuging and the supernatant drawn off for processing for detection of uric acid therein.

With the above noted small amount of serum utilized, between about 1 ml. and 1.5 ml. of supernatant will be produced in the protein separation. Because of the advantages of the invention, this quantity is adequate to permit detection of uric acid and determination of its concentration by the practice of the invention therewith.

Procedures for the separation of the serum and precipitation of protein are known and therefore, will not be further described herein.

To the supernatant formed above, a color enhancer is added to enhance the color generated by a chromogen to be subsequently added, said color when present indicating presence of uric acid. Beneficially, this color enhancer comprises a solution of nitrogen compound such as a hydrazine salt, for example, hydrazine sulfate, hydrazine dihydrochloride, hydrazine iodide and hydrazine tartrate, p-aminobenzoic acid, p-aminosalicyclic acid and phenylhydrazine hydorchloride.

The amount of color enhancer added is advantageously between about 1 and 5 milligrams per ml. of supernatant used for testing. This amount is variable over a wide range and is preferably limited by the color reaction or indication appearing in a a colorless blank fluid. A colorimeter is advantageously used to detect color changes and may be adjusted (i.e. zeroed) to compensate for a color change produced by the addition of the color enhancer, but substantial adjustments are undesirable and may result in erroneous uric acid indications.

A sequestering agent is then added to the solution of supernatant and color enhancer. The sequestering agent is preferably an organic compound selected from a group of amino carboxylic acid sequestering agents. Beneficially compounds such as ethylenedinitrillo tetraacetic acid (EDTA), tetrasodium salt; nitrilotriacetic acid (NTA); hydroxyethylenediamine triacetic acid (HEDTA), trisodium salt; diethylenetriaminepentaacetic acid (DTPA), sodium salt; di(hydroxyethyl)glycine (DEG), sodium salt; sodium pyrophosphate and combinations thereof that do not have a free amino group are used. Although the sequestering agents listed above are in the form of a sodium salt, which is preferred, other forms may be used. Between about 50 and 200 milligrams of sequestering agent per ml. of supernatant may be used, however, this quantity is not critical and may be between about 1 and 200 milligrams per ml. of supernatant used. The sequestering agent is described herein as being separate from the color enhancer, however, there may be combined in a single reagent.

The solution of supernatant, color enhancer and sequestering agent is mixed thoroughly, and a chromogenic indicator such as phosphotungstate reagent is added thereto in an amount of between about 30 and 150 milligrams per ml. of said supernatant. This addition is advantageously performed promptly after the mixing of the three components. This admixture of four components is set aside for a period of time that permits stabilization of any color change which may occur indicating the presence of uric acid. For quantities of uric acid normally found in blood that is about 5 mg. percent, color stabilization is complete in about 20 minutes. With solutions having higher concentrations, color stabilization is usually completed in about 30 minutes.

A color enhancer solution for use in the above process may be prepared by dissolving one of the previously described nitrogen compound in a suitable solvent. The quantity of compound may be between about 1 and 400 mg. percent and is preferably between 20 and 50 mg. percent. This preferred range of concentration permits the use of a convenient ratio of about 2:1 of color enhancer solution to supernatant. If concentrations in excess of about 70 mg. percent are used, some correction for color in the blank is usually necessary. A sodium acetate-aqueous solution from about 1% to saturation is a suitable solvent for the color enhancer that also gives desirable stability to the color enhancer solution.

Sensitivity of the reaction may be increased by increasing the concentration of color enhancer. However, an increase in color enhancer also appears to diminish the precision obtained and as previously mentioned, may result in color in the blank fluid.

A sequestering agent solution may be prepared by mixing one of the previously mentioned sequestering compounds with a suitable solvent. The concentration of the compound in this solution is not considered critical and may be between about 0.1 mg. percent and a saturated solution, and preferably is between about 5 and 20 grams percent.

A pH adjusting compound, preferably one that is free of interfering ions, is added to the solution to be tested to adjust the pH to between about 9 and 10. Advantageously, sodium tungstate is used for this purpose, however, other compounds imparting similar pH such as sodium hydroxide, sodium carbonate, and disodium phosphate may be used. These pH adjusting compounds may, of course, be in a solid state or included in a solution that is compatible with the test system.

The color enhancer and sequestering agent may be combined to form a single reagent. This single reagent when in an aqueous solution appears to be stable for only about 48 hours, whereas the individual solutions are stable for substantially longer periods. Considerable differences may occur between duplicates if the color enhancer solution is not accurately measured or the combined solutions have been retained too long. This combined reagent may also be formed as dry solid powder or tablets by evaporation or lyophilization.

Phosphotungstate chromogenic reagent solution is readily prepared by combining sodium tungstate (molybdenum free) in an aqueous solution with orthophosphoric acid. The combined solutions are refluxed for about 2 hours and cooled to room temperature. The resulting reagent should be refrigerated if retained as an aqueous solution. This reagent may be formed as solid tablets or powders by evaporation or lyophilization.

When detection of uric acid is to be carried out on an automatic analyzing apparatus, the novel process and composition of this invention may be readily used. The metering of solutions and time delay may be according to accepted procedures with automatic equipment. For example, the serum is diluted with a saline solution and dialysed against a recipient stream containing mixed color enhancer solution and sequestering agent solution forming an admixture which is combined with phosphotungstate reagent. This combined stream is passed through a delay coil and a determination of uric acid made with a colorimeter.

It will of course be appreciated that the order of adding the reagents to the fluid to be tested is not limited to that set forth in this description. Also, different combinations of reagents, either in the dry form or in solutions, may be used with equally effective results.

The reagents of this invention may be placed on or in a suitable carrier that may be dipped in the fluid to be tested, such as blood or serum, and the resulting color change read, for example, by comparison with a color chart. Preferably, a bibulous carrier is impregnated with a solution of the reagents and then dried. The carrier is then advantageously coated with a protective layer that prevents a large portion of the interfering substances from contacting the test reagent, particularly when the solution to be tested is whole blood.

When a bibulous carrier is employed the carrier material may be any of a variety of substances. For example, filter paper, wood strips, synthetic plastic fibrous ma-

terials, non-woven or woven fabrics and so on, may be utilized in this embodiment. The preferable bibulous material is filter paper having a thickness of about 0.01 inch to about 0.02 inch.

The invention will be further understood by reference to the following examples which describe specific preparations of solutions and detection of uric acid. These examples are not intended to be limitations on the scope of this invention which scope is properly defined in the appended claims.

EXAMPLE 1

A 5% EDTA sequestering agent solution was prepared by dissolving 50 grams of ethylenedinitrilo tetraacetic acid, tetrasodium salt in distilled water to make 1 liter. This EDTA solution was combined in a 1 to 3 ratio with a previously prepared 60% sodium tungstate pH adjusting solution. The sodium tungstate-EDTA solution formed was found to be stable at room temperature.

EXAMPLE 2

A phosphotungstate chromogenic reagent solution was prepared by dissolving 40 grams of sodium tungstate (molybdenum free) in about 300 ml. of distilled water. To this solution 32 ml. of 85% orthophosphoric acid were added and the resulting solution refluxed for 2 hours. The solution was cooled to room temperature and distilled water was added to make a volume of 1 liter. The resulting solution was stored in a refrigerator.

EXAMPLE 3

Blood samples received from 40 donors (hospital patients) were allowed to clot and serum separated by centrifuging. A portion of serum (0.2 ml.) was withdrawn from each sample and placed in individually identified test tubes containing 1.6 ml. of $\frac{1}{12}$ N sulfuric acid. The serum-acid solutions were well mixed and 0.2 ml. of a sodium tungstate solution (10%) added. The solutions combined were mixed vigorously and allowed to stand for ten minutes. A precipitate formed which was separated from the supernatant by centrifuging. A portion of the supernatant (0.5 ml.) was added to 1 ml. of a hydrazine sulfate (25 mg. percent) in sodium acetate (1%) solution and mixed well. Sodium tungstate-EDTA solution (4 ml.), prepared in Example 1, was added to the supernatant solution and mixed well. Phosphotungstate reagent (1 ml.), prepared in Example 2, was added to the supernatant solution and mixed well. The reaction solution was allowed to stand for 30 minutes at roon temperature. After 30 minutes the solutions were observed to have changed from colorless solutions to blue colored solutions, indicating the presence of uric acid. The solutions were also observed to be free of turbidity. A blank solution including all of the reagents but free of uric acid was compared with the tested solutions. The blank solution was observed to be substantially colorless whereas the solutions containing uric acid had a blue color. Concentrations of uric acid were determined by recording the absorbance of the solutions at 700 mμ with spectrophotometer. The concentration of uric acid in the sample was determined by comparing the absorbance of the solutions with a graph showing absorbance relative to concentration of uric acid that was previously prepared using solutions with known concentrations of uric acid.

EXAMPLE 4

The procedure of this example was the same as Example 3 with the exception that in place of the EDTA solution a NTA solution, 40 grams percent, was used. The color change indication with the NTA solution was substantially the same as in Example 3.

EXAMPLE 5

For this example a quantity serum was prepared as described in Example 3.

The serum was divided into five equal 10 ml. portions labeled A, B, C, D and E. Aqueous uric acid stock standard was added in varying quantities, using a microburette, representing 2 mg. percent, 4 mg. percent, 5 mg. percent, and 6 mg. percent respectively to the individual portions.

Solutions A, B, C, D and E were analyzed as described in Example 3. Recovery was calculated and it was determined 100±2.0% of uric acid was recovered by this method.

EXAMPLE 6

Solutions were prepared using serum and each of the compounds listed below which are known to interfere with the detection of uric acid by conventional methods, the concentrations being graduated up to those stated:

	Mg. percent
Ascorbic Acid	1.6
Sodium salicylate	100
Cysteine	1.6
Glucose	500

These solutions were tested according to the procedure of Example 3, and the test results regarding identification of uric acid and determination of its concentration therein were in each case observed as being the same as the results produced when a corresponding serum sample free of such interfering compounds was tested according to the same procedure.

EXAMPLE 7

The procedure of this example was the same as the procedure of Example 3 with the exception that a HEDTA (41.3%) solution was used in place of the EDTA solution. Substantially the same results were observed as in Example 3.

EXAMPLE 8

The procedure of this example was the same as the procedure of Example 3 with the exception that a DTPA (40.2%) solution was used in place of the EDTA solution. Substantially the same results were observed as in Example 3.

EXAMPLE 9

The procedure of this example was the same as the procedure of Example 3 with the exception that a DEG (41%) solution was used in place of the EDTA solution. Substantially the same results were observed as in Example 3.

EXAMPLE 10

The procedure of this example was the same as the procedure of Example 3 with the exception that a sodium pyrophosphate (5%) solution was used in place of the EDTA solution. Substantially the same results were observed as in Example 3.

EXAMPLE 11

A test device was prepared with two strips of 95% cellulose paper about .015 inch thick, ¼ inch wide and 3 inches long. One strip was impregnated with a hydrazine sulfate-sodium tungstate-EDTA solution and then dried. The second strip was impregnated with a phosphotungstate solution and dried. The strips were individually sprayed with polyvinvl pyrrolidone and bonded together along one major face. Strips prepared in this manner were dipped in fluids containing uric acid and a color change was observed that stabilized after between about 1 and 2 minutes. The strips were observed at this time and a blue color had appeared that indicated the presence of uric acid.

The foregoing description and examples show that the present invention provides a novel process and composition for the detection of uric acid. Moreover, the process of this invention is more sensitive and provides reliable results in less time than currently known processes in spite of the presence of substances which inhibit the

currently known processes. Furthermore, with the process and composition of the invention a reliable and consistent test result is obtained with a smaller sample than previously required.

What is claimed is:

1. In the process for detecting uric acid in a fluid by contacting said fluid with a phosphotungstate chromogenic reagent under alkaline conditions, the improvement comprising mixing with said fluid chromogenically enhancing amounts of a substance selected from the group consisting of a hydrazine salt, p-aminobenzoic acid, p-aminosalicylic acid and phenylhydrazine hydrochloride and an amino carboxylic acid sequestering agent prior to contacting said fluid with said phosphotungstate chromogenic reagent.

2. A process according to claim 1 in which said fluid is blood serum.

3. A process according to claim 1 in which said color enhancer is hydrazine sulfate.

4. A process according to claim 1 in which said sequestering agent is selected from the group consisting of ethylenedinitrilo tetraacetic acid, nitrilotriacetic acid, (hydroxyethyl)ethylenediaminetriacetic acid, diethylenetriaminepentaacetic acid, di(hydroxyethyl)glycine sodium pyrophosphate, and combinations and salts thereof.

5. A process according to claim 1 in which said sequestering agent is ethylenedinitrilo tetraacetic acid.

6. A process according to claim 1 in which said sequestering agent is ethylenedinitrilo tetraacetic acid, tetrasodium salt.

7. A process according to claim 1 in which said sequestering agent is nitrilotriacetic acid.

8. In a phosphotungstate chromogenic reagent a composition for detecting uric acid in a fluid the improvement which comprises the inclusion therewith of chromogenically enhancing amounts of a substance selected from the group consisting of a hydrazine salt, p-aminobenzoic acid, p-aminosalicylic acid and phenylhydrazine hydrochloride, and an amino carboxylic acid sequestering agent.

9. A composition according to claim 8 in which said amino carboxylic acid sequestering agent is a member selected from the group consisting of ethylenedinitrilo tetraacetic acid, nitrilotriacetic acid, (hydroxyethyl)ethylenediaminetriacetic acid, diethylenetriaminepentaacetic acid, and di(hydroxyethyl)glycine, sodium pyrophosphate and combinations and salts thereof.

10. A test device comprising a bibulous carrier incorporating the composition of claim 8.

11. A composition according to claim 8 in which said color enhancer sequestering agent and phosphotungstate reagent are in aqueous solution.

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U.S. Cl. X.R.

252—408