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3,585,004

## TEST COMPOSITION AND DEVICE FOR DETECTING COUPLABLE COMPOUNDS

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13 Claims

### ABSTRACT OF THE DISCLOSURE

A test composition and device which undergoes an enhanced color change when contacted by a couplable compound. The composition comprises a diazonium salt and a Lewis acid halide or a complex of a Lewis acid halide and an organic Lewis base. The device is prepared by incorporating in a carrier the diazonium salt solution and the Lewis acid halide or complex thereof with an organic Lewis base. The composition and device are used in a process for detecting couplable compounds.

This invention relates to an improved test composition and a test device which undergo a rapid and enhanced color may be measured visually or spectrophotometrically. The invention also relates to a method for preparing such a test device. More particularly, this invention relates to an improved test composition comprising a diazonium salt and a Lewis acid halide or a complex thereof with an organic Lewis base. Additionally, this invention relates to a test device incorporating the diazonium salt and the Lewis acid halide or complex thereof with an organic Lewis base and a process for the preparation thereof.

Many compounds, particularly those in body fluids such as blood or urine, are detectable by a color reaction that occurs when they are coupled with a diazo compound. One such compound which is of particular interest in the determination of a person's physical condition is bilirubin.

The detection of bilirubin in body fluids has been carried out since about 1883 when Ehrlich introduced the diazo reaction for detecting bilirubin in urine. Since that time, many liquid systems employing the diazo reaction have been used for determining bilirubin in urine and serum. These systems have introduced many variations as to the diazo compounds and other reagents used in this determination. However, such systems are primarily dependent upon the azobilirubin red-violet color which forms when bilirubin reacts with a diazonium salt, which color may be measured visually or spectrophotometrically.

Although these liquid systems result in quite accurate determinations of bilirubin, they are generally difficult to use because the diazonium salt solutions in these systems are unstable and therefore necessitate fresh preparation with each batch of fluid to be tested. Also, the time required for completion of the indicating color change is quite lengthy, the shortest being about 30 minutes and some requiring a matter of hours. Therefore, test results are not as promptly available as desired, particularly when determining bilirubin in infants.

Many of these difficulties are alleviated by a product commercially known as Ictotest®, which is in the form of a dry tablet, for determination of bilirubin as described in U.S. Pat. No. 2,854,317. In Ictotest®, the tablet comprises a diazonium salt, sulfosalicylic acid and sodium bicarbonate. To use this test, 5 drops of urine are placed on a special highly absorbent mat. The Ictotest® reagent tablet is then placed in the center of the moistened area and two drops of water flowed over the tablet. If bilirubin is present, a color reaction occurs on the mat within about 30 seconds. Although Ictotest® provides a relatively rapid

and stable system for determining bilirubin, a multiple number of components are required to complete the indicating reaction.

Test devices of the dip-and-read type, which combine specialized rapid reacting reagents with a suitable carrier, are widely utilized, particularly in the medical field. Such devices are commonly used for determining pH and detecting glucose, protein and the like in body fluids. However, when the known test systems for detecting bilirubin were combined with a suitable carrier to form a dip-and-read type device, the resulting device was unstable and yielded unreliable results.

Therefore, it is an object of this invention to provide an improved test composition which undergoes a rapid and enhanced color change when contacted by a diazo couplable compound.

Another object of this invention is to provide a unitary test device incorporating the novel test composition of this invention.

A further object of this invention is to provide a process for preparing a unitary test device which undergoes a rapid and enhanced color change when contacted by a diazo couplable compound.

Additional objects of this invention will become evident from the following description.

This invention is embodied in a novel improved test composition which undergoes a rapid and pronounced color change when contacted by a diazo couplable compound. This composition comprises a diazonium salt or a substance such as a triazene which can form a diazonium salt and a color enhancing quantity of a Lewis acid halide or a complex of a Lewis acid halide and an organic Lewis base. As used hereinafter the term diazonium salt means diazonium salts per se, or any other compound which can be reacted to form a diazonium salt.

This invention is also embodied in a test device comprising a carrier incorporating a diazonium salt and a Lewis acid halide or a complex of a Lewis acid halide and an organic Lewis base.

A stable test device may be prepared by contacting a carrier with a diazonium salt solution and drying the carrier. In preparing the diazonium salt solution, a polar solvent is used. Preferably, the composition is made up of water and an organic polar solvent such as an alcohol, a ketone, an ester and the like. An alcohol, such as methanol, ethanol and 2-propanol, is used in a ratio of at least about four parts organic solvent to one part water. The ratio of organic solvent to water is selected so that there is substantially no precipitate in the diazonium salt solution formed. Also, a high ratio of a rapidly evaporating solvent is desirable to reduce drying time of the carrier contacted with the solution.

A couplable arylamine that may be diazotized is added to the solvent first. Such an arylamine may be selected from 2,4-dichloroaniline; p-nitroaniline; p-chloroaniline; 2,5-dichloroaniline; 4-chloro-o-anisidine; 3,3'-dimethoxybenzidine; and 2-methoxy-5-nitroaniline. Other such arylamines capable of forming diazonium compounds may be used.

A suitable soluble nitrite that forms nitrous acid in an aqueous acid medium is then added to the solution. Beneficially, the soluble nitrite is sodium nitrite, potassium nitrite or calcium nitrite.

The solution may also include a stabilizer compound. Such stabilizer is beneficial in that any precipitate that appears upon the formation of the diazonium salt forms as a fine precipitate which is readily dissolved. Additionally, the stabilizer has been found to add chemical stability to the final test device. The stabilizer may be selected from a broad group of compounds, such as, aromatic or aliphatic sulfonic acid salts, sulfates or sulfonates. Examples of such stabilizers include 1,5-naphthalenedisulfonic acid, di-

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sodium salt; 2-naphthalenesulfonic acid, sodium salt; 4,4'-diamino-2,2'-biphenyldisulfonic acid, disodium salt; and sodium lauryl sulfate.

An organic acid, preferably an organic sulfonic acid, is included in the composition to provide the proper pH for the formation of a diazonium compound and the subsequent coupling reaction of this diazonium compound with the couplable compound. The organic sulfonic acid utilized may be selected from sulfosalicylic acid, sulfamic acid, hexamic, p-toluenesulfonic acid, and the like. Other known sulfonic acids and organic acids may be used.

These reagents, an organic polar solvent, an arylamine, water, soluble nitrite, a stabilizer and a sulfonic acid are combined to form the desired diazonium salt solution. Preferably, these reagents are combined in the order in which they have been mentioned, although the order of combination of the polar solvent, arylamine, water, soluble nitrite and stabilizer is not considered critical. Also, if more than one stabilizer is used, the second stabilizer may be added after the sulfonic acid.

The proportions of the reagents used to form the diazonium salt solution are preferably within the following ranges which are expressed in weight/volume percent, as appropriate:

|                       | Percent   |
|-----------------------|-----------|
| Organic polar solvent | 10-90     |
| Arylamine             | 0.02-0.5  |
| Water                 | 10-90     |
| Soluble nitrite       | 0.01-0.25 |
| Stabilizer            | 0.25-5    |
| Sulfonic acid         | 0.2-5     |

As previously mentioned, advantageously the ratio of solvent to water is maintained at 4:1 or higher to retain the diazonium salt in solution and promote the drying of the carrier. Also, the weight ratio of acid to arylamine compound is preferably maintained at about 10:1.

Surprisingly, it has been found that the color change resulting from the interaction of a diazonium salt and bilirubin is substantially enhanced when the composition includes a color enhancing quantity of a Lewis acid halide or a complex of a Lewis acid halide and an organic Lewis base.

The Lewis acid halide is advantageously selected from stannic chloride, aluminum chloride, boron trichloride and the like. The amount used is limited by the decomposition of the carrier. Beneficially, to form the test device the Lewis acid halide is included in a solution in a quantity between about 0.5 gm. and 5 gm. and preferably about 3.2 gm. per 100 ml. of solution. A suitable solvent may be used to form this solution.

Beneficially, a solid complex is formed of the Lewis acid halide and an organic Lewis base or other compound capable of sharing electrons with the Lewis acid halide. For example, compounds such as dimethyl sulfoxide, dimethyl formamide, dioxane and the like may be used. Preferably, dimethyl formamide is used to form the complex.

The complex may be formed by adding the Lewis acid halide, such as stannic chloride, to an excess of the compound used to form the complex, such as dimethyl formamide. The complex is then precipitated out of solution with a suitable organic solvent, such as chloroform, and collected. This complex may then be made up into a solution for contacting a carrier by dissolving the complex in a suitable solvent. Such solvents are preferably the solvents used to form the complex, and beneficially, in combination with a non-aqueous polar solvent such as acetone, dimethyl sulfoxide or the like.

A suitable carrier may be utilized that is capable of retaining the diazonium salt and the Lewis acid halide or complex thereof with an organic Lewis base. This carrier is preferably mounted in such a manner that it may be readily contacted by a fluid suspected of including the couplable compound to be detected. The carrier for the test device may be an integral part of a larger portion serving

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as a handle or may be a separate part that is affixed to a larger handle or handling device. Suitable carriers include, for example, a porous material such as filter paper, glass fiber paper, or polypropylene felt. The carrier may also comprise a polymeric gel which has been premixed with the test solution and dried so as to form a semi-permeable membrane structure. The gel may be formed by solvent evaporation and may include a crosslinking agent.

The overall structure of the test device is not considered critical so long as a suitable means is provided for contacting the area including the test composition with the fluid to be tested.

A test device may be prepared by impregnating a carrier with a solution of a diazonium salt and a Lewis acid halide and drying the same. The impregnated paper is preferably dried at a moderate temperature of about 65° to 70° C. in moving air. Advantageously, the temperature is not elevated excessively as this is believed to have a deleterious effect upon the diazonium compounds. The dried carrier may then be divided into small portions and affixed to a larger handle for easier handling.

Preferably, with a filter paper carrier, the carrier is first impregnated with the diazonium salt solution and dried and then with the complex of the Lewis acid halide and organic Lewis base.

The invention will be further described in the following examples which are intended to be illustrative and are not to be construed as limitations on the scope of the invention.

#### EXAMPLE 1

A solution was prepared by combining in a glass vessel, in the order listed and with continuous mixing, the following compounds:

- Methanol—20.0 ml.
- 2,4-dichloroaniline—0.20 g.
- Distilled water—20.0 ml.
- Sodium nitrite—0.1 g.
- 1,5-naphthalenedisulfonic acid disodium salt—0.6 g.
- Stannic chloride, hydrated—5.0 g.
- Sodium lauryl sulfate—1.5 g.
- Sulfosalicylic acid—2.0 g.
- Methanol—60.0 ml.

The compounds when combined were at ambient room temperature (about 23° C.) and the temperature of the solution remained at about ambient room temperature without additional temperature control throughout the formation of the solution.

A strip of filter paper (Eaton and Dikeman No. 641) was immersed in the solution prepared above and immediately removed. This paper was dried between about 65° and 70° C. for between 8 and 10 minutes with air movement over the surface of the paper. The dried paper had a cream-white color.

Bilirubin test solutions were prepared from a pathological urine sample obtained from a patient showing signs of jaundice. The urine obtained from the patient was treated with Ictotest® and a reaction was observed which indicated a high concentration of bilirubin. This urine was also assayed by a modification of the E. G. Godfried procedure for bilirubin described in Biochem. J., 28, 2056-2060, 1934, and found to have approximately 1 mg. percent bilirubin. The pathological urine was further diluted with normal urine to concentrations of 0.5, 0.25 and 0.1 mg. percent. Each solution was tested with test devices of this invention as prepared above and gradations of tan-purple colors were observed. A deeper color approaching a grey-purple, was observed for the solutions of higher concentrations.

Similarly, human blood sera were obtained from normal patients and patients showing jaundice. The bilirubin content of these sera, as determined with an Auto Analyzer®, was between 0.1 and 18 mg. percent. Test devices prepared above were contacted with the sera and colors vary-

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ing from faint pink to deep lavender were observed for concentrations of 1.0 mg. percent and higher. The color changes were proportionate to the concentration of bilirubin in the solutions.

## EXAMPLE 2

Test devices were prepared as described in Example 1 except that 1.6 g. of anhydrous stannic chloride, was used in place of the stannic chloride, hydrated. The test devices were observed to have substantially the same properties as the test devices of Example 1.

## EXAMPLE 3

A first solution was prepared by combining, in the order listed and with continuous mixing, the following components:

Methanol—20.0 ml.  
2,4-dichloroaniline—0.20 gm.  
Distilled water—20.0 ml.  
Sodium nitrite—0.10 gm.  
1,5-naphthalenedisulfonic acid, disodium salt—0.6 gm.  
Sulfosalicylic acid—2.0 gm.  
Sodium lauryl sulfate—1.5 gm.  
Methanol—60.0 ml.

A strip of filter paper (Eaton and Dikeman No. 641) was immersed in this first solution and immediately removed. This paper was dried between about 65° and 70° C. for between about 8 and 10 minutes with air movement over the surface of the paper. A second solution was prepared by combining the following components:

Dimethyl formamide—20.0 ml.  
Stannic chloride-dimethyl formamide complex—3.2 gm.  
Acetone—80.0 ml.

The dried paper was immersed in the second solution and immediately removed. The paper was dried between about 65° and 70° C. for between 8 and 10 minutes with air movement. The dried paper had a cream white color. This test device, when contacted with the test solutions of Example 1, were observed to have substantially the same color changes as noted in Example 1.

## EXAMPLES 4—10

Test devices were prepared as described in Example 3 except that first solutions were used in which one of each of the following compounds was substituted for the 2,4-dichloroaniline.

p-nitroaniline  
p-chloroaniline  
2,5-dichloroaniline  
4-chloro-o-anisidine  
3,3'-dimethoxybenzidine  
2-methoxy-5-nitroaniline

The properties of the resulting test devices were observed to be substantially the same as for the test devices of Example 3.

## EXAMPLE 11

A test device was prepared as described in Example 3 except that hexamic acid was used in place of sulfosalicylic acid in the first solution. The resulting test device was observed to have substantially the same properties as the test device of Example 3.

## EXAMPLE 12

A test device was prepared as described in Example 3 except that sulfamic acid was used in place of sulfosalicylic acid in the first solution. The resulting test device was observed to have substantially the same properties as the test device of Example 3.

## EXAMPLE 13

A test device was prepared as described in Example 3 except that p-toluenesulfonic acid was used in place of sulfosalicylic acid in the first solution. The resulting

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test device was observed to have substantially the same properties as the test device of Example 3.

## EXAMPLE 14

Strips of glass fiber paper (Gelman Type A-1306 and Type E-748) were processed in place of filter paper according to the procedure of Example 3. The resulting dried glass fiber paper had an appearance substantially the same as the paper of Example 3. When contacted by the test solutions of Example 1 color changes as noted in Example 3 were observed.

## EXAMPLE 15

In this example the procedure of Example 5 was followed except that a polypropylene felt (Polypropylene MDSE. No. PO-8110 by American Felt Company) was used in place of filter paper. The dried matrix had substantially the same color appearance and color reaction to the test solutions as observed in Example 3.

## EXAMPLE 16

A strip of Eaton & Dikeman No. 641 filter paper was passed through the first solution of Example 3 and dried between about 65° and 70° C. for about 10 minutes with circulating air. After drying, the paper was passed through a second solution comprising:

Dimethyl formamide—20.0 ml.  
Stannic chloride-dimethyl formamide complex—3.2 gm.  
Cellulose acetate—6.25 gm.  
Acetone—80.0 ml.

The paper was dried between about 65° and 70° C. for about 10 minutes in circulating air. The device so formed was contacted with the test solutions of Example 1 and a color change was noted as in Example 3.

What is claimed is:

1. In a test composition for detecting couplable compounds in aqueous fluids utilizing a diazonium salt which is specifically reactable with the couplable compound to give a chromogenic response, the improvement which comprises the addition thereto of a color enhancing quantity of a Lewis acid halide or a solid complex of a Lewis acid halide and an organic Lewis base.

2. A test composition as in claim 1 wherein the Lewis acid halide is stannic chloride, aluminum chloride or borontrichloride.

3. A test composition as in claim 1 wherein the organic Lewis base is dimethyl sulfoxide, dimethyl formamide, or dioxane.

4. A test composition as in claim 1 wherein the solid complex is of stannic chloride and dimethyl formamide.

5. A test device for detecting couplable compounds in aqueous fluids utilizing a carrier incorporating a diazonium salt which is specifically reactable with the couplable compound to give a chromogenic response, the improvement which comprises additionally incorporating in said carrier a Lewis acid halide or a solid complex of a Lewis acid halide and an organic Lewis base.

6. A test device according to claim 5 in which the Lewis acid halide is stannic chloride, aluminum chloride or borontrichloride.

7. A test device according to claim 5 in which the organic Lewis base is dimethyl sulfoxide, dimethyl formamide or dioxane.

8. A test device according to claim 5 in which the solid complex is of stannic chloride and dimethyl formamide.

9. A process for preparing a test device for detecting couplable compounds in aqueous fluids comprising incorporating a stabilized diazonium salt which is specifically reactable with the couplable compound to give a chromogenic response and a Lewis acid halide with a carrier therefor.

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10. A process according to claim 9 wherein the Lewis acid halide is stannic chloride, aluminum chloride or borontrichloride.

11. A process for preparing a test device for detecting couplable compounds in aqueous fluids comprising incorporating a stabilized diazonium salt which is specifically reactable with the couplable compound to give a chromogenic response with a carrier therefor and subsequently incorporating a solid complex of a Lewis acid halide and an organic Lewis base with said carrier.

12. A process according to claim 11 wherein the Lewis acid halide is stannic chloride, aluminum chloride or borontrichloride and the organic Lewis base is dimethyl sulfide, dimethyl formamide or dioxane.

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13. A process according to claim 11 wherein the Lewis acid halide is stannic chloride and the organic Lewis base is dimethyl formamide.

#### References Cited

#### UNITED STATES PATENTS

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JOSEPH SCOVRONEK, Primary Examiner

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23—230; 252—408

UNITED STATES PATENT OFFICE  
CERTIFICATE OF CORRECTION

Patent No. 3,585,004

Dated June 15, 1971

Inventor(s) Raymond L. Mast

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, Lines 25 - 26, Delete "may be measured visually or spectrophotometri- pound" and substitute --change when contacted by a diazo couplable compound--.

Signed and sealed this 26th day of September 1972.

(SEAL)  
Attest:

EDWARD M. FLETCHER, JR.  
Attesting Officer

ROBERT GOTTSCHALK  
Commissioner of Patents