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# STABLE BLOOD DETECTING COMPOSITION

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This invention relates to a composition which has utility in the detection of blood. Particularly the invention relates to compositions which are suitable for use in the qualitative detection and quantitative estimation of blood in body fluids such as urine, vomitus, gastric contents, cerebral spinal fluids and in feces. More particularly the invention relates to bood testing compositions wherein one component of the test is encapsulated in a colloid material.

The detection of occult blood in body fluids and feces has become an invaluable aid to the medical practitioner in the correct diagnosis of a great number of disorders. Blood is found in the gastric contents and in vomitus in conditions associated with erosion of the mucous membranes, in ulcers and in carcinomas. In the feces, the regular and frequent occurrence of occult blood is suggestive of gastro-intestinal cancer, gastric or duodenal ulcers or hemorrhoids. In these conditions, the hemorrhage is often so slight that it is not possible to detect blood by microscopic identifications of the erythrocytes (red blood cells) and a sensitive and specific chemical test for occult blood becomes invaluable. In the urine, blood cells (hematuria) or blood pigment (hemoglobinuria) is found in typhus, scurvy, purpura, pyemia, nephritis, renal calculi, as the result of a burn covering a large part of the body, by the action of various hemolytic toxins, etc.

The prior art has recognized the need for a simple, reliable test for occult blood. U.S. Patent No. 2,290,436, issued July 21, 1942, to Kamlet, U.S. Patent No. 2,799,660, issued July 16, 1957, to Nicholls and Fonner, and U.S. Patent No. 2,838,377, issued June 10, 1958, to Fonner (all assigned to the instant assignee), illustrate various test compositions which have been supplied to meet this need.

The instant inventive concept, like those of the prior art, are based on the catalytic activity of the prosthetic groups present in blood. These catalytically active substances, identified in hemoglobin, belong to the general class of hemoproteins, conjugate proteins all of which have the same prosthetic group, iron protoporphyrin or haem. This prosthetic group has the ability to catalyze the transfer of oxygen from an oxygen source to an acceptor which in turn becomes oxidized. If the acceptor is a dye precursor, colorless until it becomes oxidized and colored in its oxidized form, then the presence of the catalytic activity is indicated by color formation.

Thus the composition of this invention comprises an indicator or dye precursor plus an oxygen source. In the presence of hemoglobin, which contains the catalytic prosthetic group, the transfer of oxygen from the oxygen source to the acceptor or dye precursor will occur and the indicator will become oxidized and colored. The presence of color, then, is a means of detection of the presence of blood and the rapidity of the color change and the depth or density of the color, when compared to a set of standards is a means of the quantitative estimation of the blood present.

The instant invention relates to an improvement on the prior art techniques of testing for blood, particularly occult blood in body fluids and in other substances. It has now been found that an improved test composition results when an organic hydroperoxide is used as the oxygen source if it is first encapsulated or entrapped within microspherical bubbles of a colloid substance such as



polyvinyl alcohol, gelatin, gum arabic and carboxy vinyl polymer and combined in this form on a test stick along with a suitable indicator and buffering means. The inventive concept, therefore, comprises an organic hydroperoxide which is encapsulated in a colloid material, an indicator or dye precursor which is capable of accepting the transfer of oxygen from the organic hydroperoxide and becoming oxidized to a colored form, said transfer of oxygen being induced by the catalytic action of the prosthetic group of hemoglobin and a buffer material for maintaining the pH of the material being tested within the range of from 4 to 6.5.

In the preferred embodiment of this invention the organic hydroperoxide is encapsulated or caused to be coacervated with a colloid material so that microspherical particles which comprise tiny bubbles of the colloid material containing the organic hydroperoxide enclosed within the microspheres are impregnated or laid upon a bibulous material in stick form such as sticks of filter paper and the like. After drying the strips are then further impregnated with the indicator and the buffer. Upon immersion in the material being tested the microspherical particles rupture by the hydrolytic action of the liquid and free the organic hydroperoxide so that it becomes a source of oxygen for the reaction. Prior to immersion in the liquid to be tested the encapsulation maintains the oxygen source free from contact with other ingredients on the stick and prevents deterioration of the test composition.

Processes for the encapsulation of various oils are well known in the art. Such encapsulation processes form no part of the inventive concept herein. In general they comprise the emulsification of oils in colloid materials under suitable conditions so that droplets of the oil become surrounded by a thin film of colloid material. The emulsification is ordinarily accomplished in a water phase and after removal of this water phase, such as by centrifugation, and drying the encapsulated particles remain as integral units. Such procedures are described in U.S. Patent No. 2,800,457, issued July 23, 1957.

The inventive concept will be more clearly described with reference to the following examples which are illustrative only and are not to be considered as limiting the scope of the invention.

## EXAMPLE 1

Two grams of gum arabic were dissolved in 160 grams water, warmed to 55° C. and mixed with 8 grams cumene hydroperoxide. The resulting emulsion was kept above 50° C. and continuously stirred.

Two grams gelatin was dissolved in 160 ml. hot water and was added to the emulsion. This mixture was stirred for 15 minutes with the temperature kept above 50° C. Water was added drop by drop until 20 ml. was added. The mixture was stirred during the addition and the temperature was kept above 50° C.

The mixture was poured into 300 ml. water at 0° C. and stirred for 1 hour at a temperature not over 25° C. The pH was adjusted to 9 to 11 with 5 normal potassium hydroxide and the mixture was allowed to stand for 30 minutes. One ml. of 37% formaldehyde (adjusted to pH 9 to 11) was added and the mixture was agitated at 3° C. for 10 minutes. The mixture was filtered on a Buchner funnel and the precipitate washed with ice cold water. The precipitate was gelatinous in nature and smelled of cumene hydroperoxide.

Sticks were impregnated with a resuspension of the product and dried overnight in a 100° C. oven. The sticks were further impregnated with 4 normal, pH 4.8 citrate buffer and again dried. The impregnated area was overlaid with a solution of 20 mg. o-tolidine in 1 ml. chloroform and again dried.

That the hydroperoxide is indeed encapsulated can be shown microscopically and by an odor test. After drying the test sticks have no odor of the hydroperoxide, but on rupture with water or by heating, the odor of the hydroperoxide becomes instantly apparent. By encapsulation stable tests employing cumene hydroperoxide have been obtained. Sticks made as in Example 1 retained reactivity even after 300 hours at 75° C., while corresponding sticks made without encapsulation lost activity within 24 hours at the lower temperature of 50° C. Sticks of the encapsulated formula also retained activity for more than 96 hours at 100° C. This pronounced increase in stability on encapsulation of the hydroperoxide may be due to a decrease in the rate of decomposition of the hydroperoxide and/or a reduction of its volatility. Furthermore, discoloration of the o-tolidine indicator is prevented, since it is shielded from the hydroperoxide.

The sticks of Example 1 react with blood in urine at a dilution of 1:20,000 and with blood in water at 1:100,000.

The specificity of test sticks made with encapsulated material appears to be enhanced by the encapsulation. This can be seen in the following table:

| Type and Form of Peroxide | Minimum Concentration of Peroxidase in Water Giving Reaction, Percent | Minimum Concentration of Hemoglobin in Water Giving Reaction, Percent |
|---|---|---|
| Encapsulated cumene hydroperoxide (Example 1) | 0.0075 | 0.00015 |
| Cumene hydroperoxide (unencapsulated) | 0.00015 | 0.00015 |
| 1-Hydroxycyclohexyl hydroperoxide | 0.0000015 | 0.00075 |
| Hydrogen peroxide | 0.0000015 | 0.0003 |
| Ethyl hydrogen peroxide | 0.0000015 | 0.0015 |

1-hydroxycyclohexyl hydroperoxide, hydrogen peroxide, and ethyl hydrogen peroxides are examples of peroxides that do not possess the specificity of cumene hydroperoxide for the blood catalyzed reaction of a peroxide with an indicator. Thus encapsulated cumene hydroperoxide preparations have five times the reactivity of hydroxycyclohexyl hydroperoxide with blood but only 0.0002 the activity with peroxidase. The encapsulated cumene hydroperoxide composition has the same reactivity with blood as the uncapsulated cumene hydroperoxide preparations, but only 0.02 of the reactivity with peroxidase. Similar comparisons may be made of the other peroxides. This increased specificity becomes very important for the detection of occult blood in feces, since vegetable peroxidases may often cause false positives with the common tests for occult blood in feces. The enhancement of the specificity by encapsulating the hydroperoxide may be caused by the prevention of the breakdown of cumene hydroperoxide to hydrogen peroxide or another peroxide which does not possess the specificity of cumene hydroperoxide for the blood reaction.

## EXAMPLE 2

Diisopropylbenzene hydroperoxide was encapsulated and placed on a stick as described in Example 1 above. These sticks gave a blue color with blood in urine at a dilution of 1:20,000 and at a dilution of 1:100,000 of blood in water. As far as reactivity was concerned, these sticks were just as stable as those with cumene hydroperoxide cited in Example 1. There was even less discoloration of o-tolidine with the diisopropylbenzene hydroperoxide preparation than with the cumene hydroperoxide preparation. These encapsulated diisopropyl hydroperoxide sticks also had the marked specificity of blood noted for the encapsulated cumene hydroperoxide sticks.

## EXAMPLE 3

Example 1 was repeated using para-menthane hydroperoxide.

The sticks of this preparation reacted with a dilution of 1:20,000 of blood in urine and 1:100,000 of blood in water. They possessed the stability that has been cited for the encapsulated cumene hydroperoxide preparation in Example 1 and also the same specificity for blood.

## EXAMPLE 4

This example was conducted the same as Example 1 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned these sticks are just as stable as those of Example 1. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 1 (using undiluted suspension of the encapsulated material). The sticks of Example 4 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated cumene hydroperoxide preparations.

## EXAMPLE 5

This example was conducted the same as Example 2 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 2. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 2 (using undiluted suspension of the encapsulated diisopropylbenzene hydroperoxide). The sticks of Example 5 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated diisopropylbenzene hydroperoxide preparations.

## EXAMPLE 6

This example was conducted the same as Example 3 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 3. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 3 (using undiluted suspension of the encapsulated para-menthane hydroperoxide). The sticks of Example 6 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated para-menthane hydroperoxide preparations.

## EXAMPLE 7

Ten grams cumene hydroperoxide was emulsified with 50 grams of 10% polyvinyl alcohol (Du Pont, Elvanol 51–05) and stirred well. The temperature was kept above 60° C. Twenty-three ml. of 20% sodium sulfate (w./v.) was added dropwise to the emulsion. The emulsion was poured into 571 ml. 7% sodium sulfate (w./v.) at 19° C. and stirred. The product was collected by centrifugation or by filtration. The product was washed well with cold water and then was resuspended in 37% formaldehyde. The product was collected by centrifugation. Filter paper strips were impregnated with this product and were dried overnight in a 100° C. oven. The sticks then were further impregnated with 4 normal, pH 4.8 citrate buffer and again dried. Finally the impregnated area was overlaid with a solution of 20 mg. o-tolidine base in 1 ml. chloroform and dried in an oven for a few minutes.

The encapsulation of cumene hydroperoxide in polyvinyl alcohol could be demonstrated by an odor test. After drying the sticks had no odor of cumene hydroperoxide, but when the sticks were wet or when they were broken by abrasion, the characteristic odor of cumene hydroperoxide was immediately detectable. These sticks

also possessed the increased stability associated with encapsulation, being reactive even after 300 hours at 75° C. and after 96 hours at 100° C., while similar sticks in which the hydroperoxide was not encapsulated were unreactive after 24 hours at 50° C. The increased stability is a result of reducing the volatility and increasing the stability of cumene hydroperoxide and also increasing the stability of o-tolidine by preventing direct contact between the indicator and the hydroperoxide.

The sticks of this example give a test for blood in urine at a dilution of 1:20,000 and with blood in water at 1:100,000.

These sticks also have the specificity for blood that was cited for the sticks of Example 1.

## EXAMPLE 8

Example 7 was repeated using diisopropylbenzene hydroperoxide.

These sticks gave a blue color with blood in urine at a dilution of 1:20,000 and at a dilution of 1:100,000 of blood in water. As far as reactivity was concerned these sticks were just as stable as those with cumene hydroperoxide cited in Example 7. There was even less discoloration of o-tolidine with the diisopropylbenzene hydroperoxide preparation than with the cumene hydroperoxide preparation. These encapsulated diisopropyl hydroperoxide sticks also had the marked specificity for blood noted for the encapsulated cumene hydroperoxide sticks.

## EXAMPLE 9

Example 7 was again repeated using para-menthane hydroperoxide.

The sticks of this preparation reacted with a dilution of 1:20,000 of blood in urine and 1:100,000 of blood in water. They possessed the stability that has been cited for the encapsulated cumene hydroperoxide preparation in Example 7 and also the same specificity for blood.

## EXAMPLE 10

This example was conducted the same as Example 7 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 7. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 7 (using undiluted suspension of the encapsulated material). The sticks of Example 10 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated cumene hydroperoxide preparations.

## EXAMPLE 11

This example was conducted the same as Example 8 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 8. There is even less discoloration of the indicator. The sticks appear to be slightly more sensitive than those of Example 8 (using undiluted suspension of the encapsulated diisopropylbenzene hydroperoxide). The sticks of Example 11 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood associated with the other encapsulated diisopropylbenzene hydroperoxide preparations.

## EXAMPLE 12

This example was conducted the same as Example 9 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 9. There is even less discoloration of the indicator. The sticks appear to be slightly more sensitive than those of Example 9 (using undiluted suspension of the encapsulated para-menthane hydroperoxide). The sticks of Example 12 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood associated with the other encapsulated para-menthane hydroperoxide preparations.

## EXAMPLE 13

First, 2 gms. gum arabic was dissolved in 160 ml. hot water and the temperature was adjusted to about 60° C. Then, 8 gms. cumene hydroperoxide was added to the solution with rapid stirring. Two grams purified pig skin gelatin was dissolved in 160 ml. hot water, and this solution was added to the emulsion. The resulting suspension was stirred vigorously for 15 minutes at a temperature above the melting point of the gelatin (about 60° C.). Next the pH was adjusted to 5 with 20% NaOH. Fifty-five ml. of water at 60° C. was added to the suspension, and the pH was adjusted to 4.4 with 1 normal $H_2SO_4$. To the suspension was added 0.4 gm. 37% formaldehyde, and the entire mixture was placed in an ice-$CaCl_2$ bath. When the temperature had dropped to 10° C., the suspension was allowed to stand for ½ hour. Then the pH was adjusted to 9 with 20% NaOH. The resulting suspension was centrifuged for 1 hour at 2,000 r.p.m. The product which came down was a white, gelatinous substance which smelled strongly of cumene hydroperoxide.

This gelatinous substance was impregnated onto strips of filter paper, which were placed in a 75° C. oven and allowed to dry for approximately 16 hours. The resulting strips of filter paper, which no longer carried the odor of cumene hydroperoxide, were dipped in 4 normal pH 4.8 citrate buffer, and placed in the oven again to dry for about 3 or 4 hours. Finally the reagent area of the filter paper strips was impregnated with a solution of o-tolidine base (20 mg./ml.) in chloroform. These strips were dried for a few minutes at 75° C., cut, and bottled with a desiccant.

The encapsulation of cumene hydroperoxide could be shown by an odor test. After drying the sticks had no odor of cumene hydroperoxide, but when the sticks were wet or when they were broken by abrasion, the characteristic odor of cumene hydroperoxide was immediately apparent. These sticks also possessed the increased stability associated with encapsulation noted in Example 1, being reactive even after 300 hours at 75° C. and after 96 hours at 100° C., while sticks in which the hydroperoxide was not encapsulated were unreactive after 24 hours at 50° C. The increased stability is a result of reducing the volatility and increasing the stability of cumene hydroperoxide and also increasing the stability of o-tolidine by preventing direct contact between the indicator and the hydroperoxide. The sticks prepared according to Example 7 react with blood in urine at a dilution of 1:20,000 and with blood in water at 1:100,000. There is also the marked specificity for blood in contrast to vegetable peroxidase noted in Example 1.

## EXAMPLE 14

This example was conducted the same as Example 13 using, however, diisopropylbenzene hydroperoxide.

These sticks gave a blue color with blood in urine at a dilution of 1:20,000 and at a dilution of 1:100,000 of blood in water. As far as reactivity was concerned, these sticks were just as stable as those with cumene hydroperoxide cited in Example 13. There was even less discoloration of o-tolidine with the diisopropylbenzene hydroperoxide preparation than with the cumene hydroperoxide preparation. The encapsulated diisopropylbenzene hydroperoxide sticks also had the marked specificity for blood noted for the encapsulated cumene hydroperoxide sticks.

EXAMPLE 15

This example was conducted the same as Example 13 using, however, para-menthane hydroperoxide.

This preparation with encapsulated para-menthane hydroperoxide performed like the encapsulated cumene hydroperoxide preparation of Example 13 with regard to stability, to sensitivity, and to specificity.

EXAMPLE 16

The example was conducted the same as Example 13 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned, these sticks are just as stable as those of Example 13. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 13 (using undiluted suspension of the encapsulated material). The sticks of Example 16 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated cumene hydroperoxide preparations.

EXAMPLE 17

This example was conducted the same as Example 14 using, however 1/10 dilution of the encapsulated material in water.

As with the encapsulated cumene hydroperoxide preparations, using 1:10 of the encapsulated diisopropylbenzene hydroperoxide material appeared to give more reactive sticks. The sticks of Example 17 reacted with dilutions of blood in urine of 1:30,000 to 1:50,000 and with blood in water of 1:100,000. The sticks were just as stable as those of Example 14 and there was even less discoloration of the o-tolidine. They also possessed the specificity for blood as mentioned for the other preparations.

EXAMPLE 18

This example was conducted the same as Example 15 using, however, 1/10 dilution of the encapsulated material in water.

This preparation with encapsulated para-menthane hydroperoxide performed like the encapsulated cumene hydroperoxide preparation of Example 16 with regard to stability, to sensitivity and to specificity.

EXAMPLE 19

First, 4 gm. purified pig skin gelatin was dissolved in 40 ml. hot water, and the temperature was kept above 50° C. Then 1 ml. cumene hydroperoxide was added to the solution with rapid stirring. To the resulting colloidal suspension was added dropwise 16 ml. 20% sodium sulfate (w./v.). The suspension was agitated for 15 minutes at a temperature above 50° C. This suspension was poured into 400 ml. 7% sodium sulfate (w./v.) at 19° C. with constant stirring. Then the mixture was centrifuged for 1 hour at 2,000 r.p.m. A gelatinous layer was skimmed off the top and resuspended in 20 ml. 37% formaldehyde. This suspension was diluted to 800 ml. and centrifuged again for 1 hour at 2,000 r.p.m. The product was a white, gelatinous substance.

This gelatinous substance was impregnated onto strips of filter paper which were placed in a 75° C. oven and allowed to dry for approximately 16 hours. The resulting strips of filter paper, which no longer carried the odor of cumene hydroperoxide, were dipped in 4 normal pH 4.8 citrate buffer and placed in the oven again to dry for about 3 or 4 hours. Finally the reagent area of the filter paper strips was impregnated with a solution of o-tolidine base (20 mg./ml.) in chloroform. These strips were dried for a few minutes at 75° C., cut, and bottled with a desiccant.

That the hydroperoxide is indeed encapsulated can be shown microscopically and by an odor test. After drying the test sticks have no odor of the hydroperoxide, but on rupture with water or by heating, the odor of the hydroperoxide becomes instantly apparent. As cited in Example 1, the stability of sticks containing cumene hydroperoxide is greatly increased by this encapsulation. The sticks are stable for more than 300 hours at 75° C. and for more than 96 hours at 100° C., while similar sticks without encapsulation of the hydroperoxide are unreactive after 24 hours at 50° C. The increase in stability is a result of both reducing the volatility and increasing the stability of cumene hydroperoxide and of preventing discoloration of o-tolidine since there is no direct contact between the hydroperoxide and the indicator. The sticks of Example 19 react with blood in urine at a dilution of 1:20,000 and with blood in water at 1:100,000. The sticks of Example 8 possess the same specificity for blood cited in Example 1.

EXAMPLE 20

This example was conducted the same as Example 19 using, however, diisopropylbenzene hydroperoxide.

These sticks gave a blue color with blood in urine at a dilution of 1:20,000 and a dilution of 1:100,000 of blood in water. As far as reactivity was concerned these sticks were just as stable as those with cumene hydroperoxide cited in Example 19. There was even less discoloration of o-tolidine with the diisopropylbenzene hydroperoxide preparation than with the cumene hydroperoxide preparation. The encapsulated diisopropylbenzene hydroperoxide sticks also had the marked specificity for blood noted for the encapsulated cumene hydroperoxide sticks.

EXAMPLE 21

This example was conducted the same as Example 19 using, however, para-menthane hydroperoxide.

This preparation with encapsulated para-menthane hydroperoxide performed like the encapsulated cumene hydroperoxide preparation of Example 19 with regard to stability, to sensitivity, and to specificity.

EXAMPLE 22

This example was conducted the same as Example 19 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned these sticks are just as stable as those of Example 19. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 19 (using undiluted suspension of the encapsulated material). The sticks of Example 20 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated cumene hydroperoxide preparations.

EXAMPLE 23

This example was conducted the same as Example 20 using, however, 1/10 dilution of the encapsulated material in water.

As far as reactivity is concerned these sticks are just as stable as those of Example 20. There is even less discoloration of the indicator. The sticks appear to be slightly more reactive than those of Example 20 (using undiluted suspension of the encapsulated material). The sticks of Example 23 react with blood in urine at a dilution of 1:30,000 to 1:50,000 and with blood in water at a dilution of 1:100,000. They also possess the specificity for blood noted for the other encapsulated diisopropylbenzene hydroperoxide preparations.

EXAMPLE 24

This example was conducted the same as Example 21 using, however, 1/10 dilution of the encapsulated material in water.

This preparation with encapsulated para-menthane hydroperoxide performed like the encapsulated cumene

hydroperoxide preparation of Example 21 with regard to stability, to sensitivity, and to specificity.

## EXAMPLES 25

To illustrate another method of preparation of the encapsulated hydroperoxides to which this invention is directed, the following example is given:

There was mixed in a mortar 0.75 gm. of a high molecular weight carboxy vinyl polymer, commercially available under the trade name Carbopoal, and 8 ml. of cumene hydroperoxide. 25 ml. of water was added in increments, slowly. One gm. of gelatin was dissolved by heating in 50 ml. of a 4 normal citrate buffer having a pH of 4.8 and this solution was added to the mortar. The material was mixed well and 2 ml. of 37% formaldehyde solution and 0.5 ml. of a 5% aerosol solution was added. These ingredients were thoroughly mixed and the emulsion was run through a hand homogenizer.

Sticks were coated with this mixture and were dried at 67° to 75° C. for 16 hours. The coated portion of the sticks was then immersed in a solution of 160 ml. of orthotolidine in 8 ml. of chloroform. The sticks were then dried in an oven at 75° C.

These test sticks reacted with blood in urine at a dilution of 1:20,000 and with blood in water at a dilution of 1:100,000.

## EXAMPLE 26

In addition to the stick tests described above, there is also contemplated another form of the test for blood in body substances, particularly feces. In this concept of the invention a square of paper such as filter paper is impregnated by dipping in a 10% solution of any of the encapsulated peroxides as described in the examples above. The paper is then dried and is ready for use.

*Tablet A*

A tablet is prepared having the following formulation:

| | G. |
|---|---|
| O-Tolidine | 10 |
| Trisodium citrate (dihydrate) | 32.6 |
| Anhydrous citric acid | 7.4 |
| Lactose | 100 |
| Talc | 2 |
| Aerosol/100 ml. chloroform | 5 |

*Tablet B*

If desired the following formulation may be used:

| | | |
|---|---|---|
| O-Tolidine·2HCl | g. | 15 |
| Trisodium citrate | g. | 32.6 |
| Citric acid | g. | 7.4 |
| Lactose | g. | 95 |
| Dried bovine plasma | g. | 5 |
| Talc | g. | 2 |
| Sodium bicarbonate | g. | 5 |
| D and C Red No. 35 | mg. | 100 |

In preparing tablets A and B, the powder is mixed in dry form suspended in 100 ml. of 95% alcohol and granulated by forcing through a fine mesh screen. The granulation is then dried for ½ hour and made into tablets.

In carrying out this test a sample of the feces or other body substance to be tested for the presence of occult blood is smeared on the peroxide paper. The tablet, either A or B, is placed on the smear and two drops of water is added. The appearance of blue color on the wetted portion of the paper surrounding the tablet is an indication of the presence of occult blood in the sample.

In addition to the orthotolidine indicator or dye precursor which is used in the examples above, it will be appreciated that any indicator material which is capable of accepting oxygen and being oxidized to a colored dye in the presence of the oxygen source and the prosthetic group present in the hemoglobin of blood may be used. For example, such indicators as aniline and its derivatives, o-tolidine, o-toluidine, p-toluidine, o-phenylenediamine, N,N'-dimethyl-p-phenylenediamine, N,N'-diethyl-p-phenylenediamine, benzidine, di-anisidine, o-cresol, m-cresol, p-cresol, alpha-naphthol, beta-naphthol, catechol, guaiacol, pyrogallol, etc. may be used.

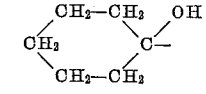
In addition to the citrate buffer described in connection with the examples above, other buffers such as tartrate, phosphate, phthalate, acetate and mixtures of these may be used. It is essential that the buffer selected maintain the pH within a range in which the indicator material changes color upon oxidation. Normally, this will be within a pH of about 4 to 6.5.

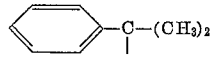
The organic hydroperoxides which may be used in accordance with the inventive concept have the formula:

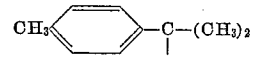
R—O—OH

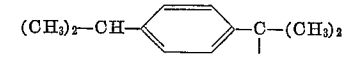
wherein R is selected from the group consisting of the following:

$CH_3CH_2$—; $(CH_3)_3C$—









The major active ingredients and the operable and preferred amounts of each which are utilized in the test compositions of this invention are set out in tabular form below.

TABLE I

| Constituent: | Preferred range (parts by wt.) |
|---|---|
| Indicator dye | 15–25 |
| Organic hydroperoxide (encapsulated) | 20–70 |
| Buffer | 50–400 |

To summarize briefly, the instant invention relates to test compositions for the qualitative detection and the quantitative estimation of occult blood. The compositions comprise an organic hydroperoxide as an oxygen source which is encapsulated in a colloid material in the form of entrapped particles within a microspherical shell. A buffer for maintaining the pH of the material being tested to one within a range of from about 4 to about 6.5 and an indicator material which is capable of accepting the transfer of oxygen from the organic hydroperoxide by the catalytic action of the prosthetic group of hemoglobin. The test compositions are preferred impregnated upon a bibulous carrier such as a filter paper strip, a compression of cellulose fiber, a compression of ground cellulose and the like. There is also contemplated within the scope of this invention a test composition which is in two parts; one part being a square or mat of cellulose fiber upon which is impregnated the encapsulated organic hydroperoxide; the other part of which is a tablet containing the indicator and a solid acid acting buffer material which contains the usual inert excipients, lubrication aids and the like. In this concept, specimen to be tested is placed on the square of cellulose material, the tablet is placed on the specimen and two drops of water are placed on the tablet. The appearance of the color in the area of the test paper surrounding the tablet indicates a positive reaction.

What is claimed is:

1. A test composition for the detection of occult blood which comprises a bibulous strip of cellulose fiber which has been impregnated with an organic hydroperoxide se-

lected from the group consisting of cumene hydroperoxide, diisopropylbenzene hydroperoxide and para-menthane hydroperoxide, which has been encapsulated in microspheres of a colloid, a buffer for maintaining the pH of the material being tested to one within the range of about from 4 to 6.5 and an indicator material which is capable of accepting the transfer of oxygen from the organic hydroperoxide by the catalytic action of the prosthetic group of hemoglobin and becoming colored thereupon.

2. A test composition in accordance with claim 1 wherein said organic hydroperoxide is cumene hydroperoxide.

3. A test composition in accordance with claim 1 wherein said organic hydroperoxide is diisopropylbenzene hydroperoxide.

4. A test composition in accordance with claim 1 wherein said organic hydroperoxide is para-menthane hydroperoxide.

5. A test composition in accordance with claim 1 wherein said indicator material is selected from the group consisting of aniline derivatives.

6. A test composition in accordance with claim 1 wherein said indicator material is orthotolidine.



7. A test composition for the detection of occult blood which comprises from 20 to 70 parts by wt. of an organic hydroperoxide selected from the group consisting of cumene, diisopropylbenzene and para-menthane hydroperoxides, said organic hydroperoxide being encapsulated within microspheres of a colloid material, from 15 to 25 parts by wt. of an indicator dye which is capable of accepting the transfer of oxygen from the organic hydroperoxide by the catalytic action of the prosthetic group of hemoglobin and becoming oxidized into its colored form thereby, and from 50 to 400 parts of a buffer material capable of maintaining the pH of the material being tested to one within the range of from 4 to 6.5.

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