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DRY REAGENT FOR TESTING

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This invention is a dry reagent for the direct and immediate testing, without external heating, for sugar, of bodily and other solutions, as blood and urine, particularly the latter. The ingredients in dry crystalline or powder form are mixed dry and in any order and the product is dry. It is in powder or tablet form ready, without heat other than that of the reaction resulting when a drop of the solution falls upon it, to give the test on the instant.

The invention is based on the well-known Nylander test for sugar in urine by the reduction of a bismuth compound in the presence of a caustic alkali by sugar when present in the urine. Particles of the metal bismuth, freed in this way, being black are readily recognized and prove the presence of sugar. This, i. e. the Nylander test, is a wet test, the ingredients in solution being mixed with the urine and subjected to external heat. But it was natural to suppose that a dry mixture of the bismuth compound and the caustic alkali would react similarly with urine and would have the considerable advantages of dry reagents over solutions and, in fact, the dry mixture of a bismuth salt, bismuth subnitrate, and a caustic alkali, as sodium hydroxide, gives a black color with a solution of dextrose, the heat evolved in the moistened powder by the reaction of sodium hydroxide and water being sufficient to insure the reaction between the bismuth salt and sodium hydroxide, without the boiling necessary in the wet test. However, the fact that such a dry test will work when the materials are freshly mixed is not enough to demonstrate the practicability of the test under the exacting conditions of general use, where individuals may make their own unsupervised tests and where indefinitely old reagent—perhaps left exposed in damp air—may be the medium for the urine test. On the contrary, a reagent of this class to be of permanent practical value must be capable of giving immediate, sharply clear and conclusive results whenever it is desired to use the reagent, whatever the lapse of time since it was made up. The test must be decisive and inability to meet this critical requirement no doubt explains why this very simple test never became popular among physicians and laymen, in spite of its decided advantages over all other tests for sugar (Fehling's, Benedict's, Haine's and even Nylander's itself when used in solution).

The dry mixture of a bismuth compound and sodium hydroxide is unstable and decomposes slowly in the presence of air, light, moisture, dust, etc., or any of them, so that after a few months, three to six under ordinary conditions and less in hot countries or where the air is moist, it cannot be relied upon to give an immediate and certain test, and any blackening which does in time occur may be due to the normal reducing power of the solution and not to sugar, so that the test becomes inconclusive and the reagent useless. Speed, always and evidently desirable, thus becomes essential in testing low percentage glucose solutions. For example, in urine, the presence of certain non-sugar constituents (as a high percentage of uric acid or etc.) in the tested urine may give the sugar test in the absence of glucose, but it would take five or ten minutes or more for the darkening to appear.

Furthermore, the bismuth compound-sodium hydroxide powder is extremely hygroscopic, so that if it is left in the open air when the atmosphere is humid, a simple mixture of a bismuth compound and sodium hydroxide would hardly resist more than half an hour or an hour without melting and becoming useless, and even where this did not occur, inadvertently leaving the bottle or etc. containing the mixture open might result in mistaken reliance by the person making the test on results vitiated by the condition of the reagent.

This is recognized in the German patent to Fornet, No. 592,923, published February 19, 1934, in which it is proposed to meet this defect by hermetically enclosing the powder in a capsule of hyaloid cellulose, a waterproof material, until pierced at the moment of use.

The present invention, when considered in its entirety lies in removing or minimizing the critical defects in the dry bismuth compound-caustic alkali mixture, viz., instability and the excessive hygroscopic characteristic, which have been pointed out, and in speeding up the sugar reducing reaction and obtaining the maximum total heat production in minimum time, and the sharpest test indication. This is done by adding other ingredients whose nature will hereinafter be made to appear and by the choice of the bismuth compound.

In studying bismuth salts and other bismuth compounds over a long period and in making many tests with them, I have ascertained that a dry mixture of a bismuth compound and caustic alkali may be made much more stable by the addition of minute amounts of ingredients of a certain class which I have found to have the effect of counteracting the tendency of the bis-

muth compound to break down and preventing the premature dehydrating action of the alkali hydroxide. The stabilizer here referred to is selected from the group consisting of lead and magnesium compounds. Bismuth is ordinarily associated with certain other metals, arsenic, antimony, iron, silver, lead, magnesium, present in metallic form as impurities, and I found it to be true that chemically pure bismuth subnitrate is not as stable as impure bismuth subnitrate and that the like difference is still more apparent in bismuth subcarbonate. By treating the bismuth with nitric acid, thus obtaining the metallic impurities as nitrates or other salts and by a series of tests, I ascertained that the stabilizing effect in the case of both the bismuth salts referred to was due to the lead and the magnesium, the lead having more effect in this respect than the magnesium and both together having several times the effect of either alone, the criterion being the effect of salts or other compounds of these metals when used with the respective bismuth salts on change of color of the salt under conditions of temperature, light, lapse of time, etc., which would tend to cause change of color. Accordingly, I stabilized the mixture of the bismuth compound and caustic alkali by the addition of minute proportions, large amounts not being necessary, with reference to the total weight of the reagent, in the case of each stabilizing compound, of .05% by weight, more or less, of a compound (neutral in color) of each of these metals, which were found to have a negative catalytic effect in the decomposition of bismuth salts or other bismuth compounds. Magnesium oxide and magnesium carbonate I regard as the best magnesium compounds for this use, both being very light, voluminous and flocculent. I have also used magnesium nitrate and magnesium sulphate successfully, and I believe from my investigations that any other compound of magnesium would so far possess the requisite characteristics as to serve the purpose. I have tried with success each of a number of lead compounds, among them lead nitrate, lead acetate, lead oxide and lead sulphate and I am satisfied that it is the metal, lead, which gives the lead compound the stabilizing effect. I believe, therefore, that any compound of lead, whether or not organic, not disqualified by having a color sufficiently pronounced to obscure the test, as in case of red lead, lead sulphide, etc., would be acceptable for this purpose and would be understood by a competent chemist to be so. As in the case of the bismuth salt, so in that of the mixture, the lead has a greater stabilizing effect than the magnesium and both the lead compound and the magnesium compound together several times the effect of either alone. I have found further that these compounds are effective in stabilizing bismuth compounds in a mixture of this character against each and all of the adverse factors already named, and that they are especially so in the case of bismuth compounds of water-free character to which further reference will be made in the course of this specification. Even if present in amounts largely in excess of those named, they do not interfere with the normal reactions of the bismuth compound and do not have any injurious effect on the normal or abnormal constituents of urine. It is apparent that when present in such amounts they should be anhydrous.

Proof of enhanced stability can be had only by tests at long intervals of a given reagent. Such prolonged tests are back of this invention and application. I have ascertained in this way that a powdered reagent of this nature, viz., a mixture of bismuth oxychloride, sodium hydroxide, magnesium oxide and anhydrous lead nitrate, after being kept for a year, reacted in a test of urine immediately and with precision just as when it was newly compounded. And there is no reason to suppose that it would not continue indefinitely in the like unimpaired condition. This illustrates one aspect of my invention.

My belief as to the way in which these added compounds act is that the voluminous magnesium compound has an effect at least partly mechanical and that the lead compound and probably the magnesium compound also effect the chemical operation in a manner which I do not altogether understand, but which slows up, defers or prevents interaction of the ingredients of the powder, particularly in the presence of moisture in the pre-test powder, which is to say that the added compounds act as stabilizers and also as negative catalysts.

In referring above to the bismuth compounds, I have distinguished between compounds having water associated with them as water of constitution or as water of crystallization, and anhydrous compounds of bismuth which I describe as water-free and which do not yield water even when heated. This is a distinction of importance and only bismuth compounds of the latter character should be used in my reagent, since a dry bismuth compound containing water in its molecule, such as bismuth subnitrate and bismuth sbucarbonate, is readily attacked by sodium or potassium hydroxide. This is illustrated by the fact shown by actual tests of a powder containing subcarbonate of bismuth,

$$CO_3(BiO)_2+1/2(H_2O)$$

and caustic alkali, which when first made up gave a successful test for glucose in urine, but after three months became a mass of lumps capable when moistened of giving but very little heat and useless for sugar tests except with high percentage solutions containing 1% or more of sugar, whereas in urine .2% is pathological. Bismuth salts containing water thus become in time unfit for delicate sugar tests.

The water-free compound of bismuth which I find much the best for this purpose is bismuth oxychloride, BiOCl, which in making the test is changed to a fresh and therefore active oxide of bismuth, probably Bi_2O_3, which is readily reduced by sugar in the urine (or other solution) to metallic bismuth or other bismuth indication. Bismuth oxychloride is a very fine, light powder, not hygroscopic at all, and water free, all of which characteristics are of special advantage in my reagent. It is a white powder. Bismuth chloride, $BiCl_3$, on the contrary is highly hygroscopic, and though it is anhydrous, its use is excluded by practical considerations. It must not be inferred, however, that bismuth oxide, Bi_2O_3, or other bismuth oxide, is suitable for use initially in a sugar testing reagent. Although this compound is entirely free of water, it has a very unfavorable influence on the sensitiveness and speed of the reaction. And this is true also where the oxide is formed slowly by the breaking down of a different compound of bismuth in a dry testing reagent of this class. In order to be quickly and easily reduced to black metallic bismuth, the bismuth oxide must be as freshly prepared as possible, ideally at the instant of per-

forming the test. Oxychloride of bismuth and other anhydrous bismuth salts, as bismuth sulplate, borate, and phosphate act in this way producing a fresh and highly reactive oxide, the reducing action of the sugar being much more energetic under these conditions, this enhanced reactivity and reducibility being a common property of metallic oxides at the moment of their formation.

The other characteristic of a dry reagent of this class which incapacitates it for safe practical use in all hands indiscriminately is that it is extremely hygroscopic. This, by adding moisture, increases its natural instability and creates conditions unfavorable to the heat producing reaction which is the basis of the test, as well as to the reactions resulting in the reduction of the bismuth compound. While my reagent is not completely non-hygroscopic, I have been able to reduce its hygroscopic character very materially, so that the reagent can be exposed to the open air for a substantial time (twenty-four hours in dry air) without disqualifying it for use for testing purposes. The reagent would react thereafter as well, if not so quickly as before. I have found that sodium silicate or potassium silicate, when added in substantial amount to the bismuth salt-caustic alkali powder (with or without a further stabilizer), both have this beneficial effect, although themselves hygroscopic, and act also in other vital ways about to be stated. They both have capillary structure and are soluble. By capillary structure is meant that the ingredient is characterized throughout its mass by a multiplicity of capillaries, i. e. capillary pores, canals or crevices. The action is understood to be by interfacial tension. Both the dry silicates named may be obtained in the open market. Each is understood to act in four ways. (1) It makes the mixture less hygroscopic. (2) It lessens the contact or intimacy of the contact, between the other ingredients, thus by mechanical dilution diminishing also their reaction while awaiting use and further stabilizing the powder. (3) It is a reaction-accelerator. It distributes the solution in making the test, i. e., having capillary structure it enables the small drop of solution (urine or etc.), by which it is capable of being wet, to penetrate immediately by a multiplicity of paths the entire mass of the small portion of powder used in the test (about 0.2 of a gram) permeating on the instant every particle and thus carrying out the reaction at the same time in every part of the mass thereby attaining in the least time the highest temperature that can be obtained with a given amount of powder, and (4) giving the sharpest definition in the test indication as well as the quickest.

The added ingredient is a material compatible with the reaction or reactions which the test involves and substantially inert to the other materials of the mixture in its substantially dry state. This means that the material is not an oxidizing agent, or a reducing agent or acidic in character. This fact assists in the performance of functions (1) and (2).

In performing function (3) the ingredient overcomes, by virtue of its capillary structure, the fundamental difficulty inherent in the simple bismuth compound-caustic soda mixture due to insolubility of the bismuth compound and of reaction products. Both bismuth hydroxide, and bismuth oxide formed when the powder being hygroscopic is moist, as well as in the test reaction itself, are not capillary in their structure and are insoluble as well and so present a sort of wall or insulation localizing the reaction by delaying or preventing progress of the moisture of the solution to the non-proximate particles of the powder, thereby reducing the extent of the reaction, the promptness of it and the amount and speed of heat production frequently below the point essential to the test, so that a negative result is meaningless and the reagent cannot be relied on. The addition of this compound having a capillary structure substitutes positive values at each of these points for the negative disadvantages inherent in the bismuth salt-alkali mixture, and assists in making it safe to rely on the reagent. Each of the functions above enumerated contributes to the efficacy of the reagent and all are important. As to function (4): This is the direct consequence of the realization of function (3), but there is a further increase or accentuation of sharpness of the test indication believed to be due to the power of the soluble sodium silicate or potassium silicate to protect colloids against coagulation. This preserves the very intense color characteristic found even in very small quantities of colloids, as against absence or faintness of color in the same quantities when coagulated and no longer colloidal. Prevention of coagulation of the metallic bismuth, which at the instant of its formation by reduction of its oxide by sugar is a colloid, is thus insured so that the test is exceptionally sharp even with low percentage of sugar. This ingredient thus has a stabilizing effect in the dry reagent and acts also during the test as a negative catalyst. As already indicated in the specification and claims, it may be used with the dry bismuth salt-caustic alkali mixture even without the lead compound or the magnesium compound or both of them.

Soluble silicates, in particular sodium and potassium silicates, are preferred to other materials since they can perform all four of the listed functions very effectively and give the most rapid and sharpest test of any material or compound of which applicant is aware, being in these respects notably superior.

Up to this point little has been said as to the relative amounts in which the principal ingredients are to be used. The fact is that this is dictated in a general way by the nature of the ingredients and by the task to be performed. The percentages of the respective materials will be varied according to the solution to be tested, the amount of sugar, if any, normally in the solution and like factors. For testing urine, the powder reagent is made up to be sensitive to not less than about 0.1%. This is sufficiently sensitive for the sugar test, but not so sensitive as to give the black sugar test indication immediately in response to non-sugar constituents having reducing power. Putting in more of the bismuth salt which being insoluble tends to cover the black in the test makes the reagent less sensitive, as is necessary in testing blood which normally has approximately 0.1% of sugar, so that the reagent, in this case, should not respond to so little as 0.1% of sugar. It will be apparent that in compounding the reagent account must always be taken of the insolubility of the bismuth compound, which if present in undue amount tends to cover the black particles of the free bismuth and so obscure the test. Account must also be taken of the amount of any other insoluble ingredient, and the total insoluble content of the reagent must be kept below the point at which

it would obscure the test indication to an extent not permissible for the particular solution to be tested. Whether, or not specified in particular claims, it is to be understood, also, that materials which by their color and amount would obscure the test are excluded.

Proportions by weight which I have found in actual practice to be effective in the dry reagent for a sugar test of urine are as follows: bismuth salt (bismuth oxychloride) 12.5%; caustic soda 56.5%; magnesium oxide or magnesium carbonate 0.05%; nitrate of lead 0.05%; and the balance sodium silicate. Testing reagent of this composition and proportions is still fully effective and reliable many months after it was made up. It is to be understood that the proportions just given, while suggestive and while indorsed by experience are approximate and are not to be taken as invariable, but are to be modified according to the nature of the particular solution to be tested and other special circumstances as will be apparent to a chemist. The ingredients are most readily mixed in the form of powder and in any order. None of the ingredients is wet; all are dry and not too coarse in the case of the silicate or capillary ingredient to have a capillary action, and not too coarse to lose the power to counteract hygroscopic characteristic of the mixture. No difficulty is experienced on this score, however, since the dry sodium silicate and the dry potassium silicate on sale in the open market have capillary structure and are suitable for applicant's purpose. Compacted into tablets the reagent is less sensitive, and its use as a powder is as a rule preferred.

In making the urine test the reagent (about .2 of a gram), when moistened with a drop of urine, acts instantly, in one or two seconds, that is, and marks a decided advance in that important respect, the rapidity of the test excluding as the cause of the indication everything but sugar. The reaction is very sharp giving under 0.1% of sugar only a slight gray color whereas with 0.2% a strong black color which never fails to appear and which is recognized without difficulty or possibility of error is developed. The reagent is stable and would not give any yellow, gray or black coloration, other than the test.

I claim:

1. A dry reagent for testing urine for sugar adapted to react to a drop of urine to test it without the application of heat other than that of the reaction itself, containing a water-free bismuth salt, caustic alkali, and sodium silicate.

2. A dry reagent for testing urine for sugar adapted to react to a drop of urine to test it without the application of heat other than that of the reaction itself, containing a water-free bismuth salt, caustic alkali, and potassium silicate.

3. A dry reagent for drop contact testing of solutions for sugar comprising a mixture containing an anhydrous bismuth salt, caustic alkali, and a lead compound and a magnesium compound both neutral in color and serving as stabilizers for the mixture prior to use.

4. A dry reagent for drop contact testing of solutions for sugar comprising a mixture containing an anhydrous salt of bismuth, caustic alkali, and a mixture-stabilizing magnesium compound.

5. A dry reagent for testing urine for sugar, on contact without the application of external heat, containing bismuth oxychloride, and caustic alkali, a magnesium compound selected from the group consisting of the oxide and the carbonate and nitrate of lead, and sodium silicate.

6. A dry reagent for drop contact testing of solutions for sugar, comprising a mixture containing an anhydrous bismuth salt, caustic alkali, a stabilizer for said mixture selected from the group consisting of lead and magnesium compounds, and a soluble silicate.

7. A dry reagent for drop contact testing of solutions for sugar, comprising a mixture containing an anhydrous bismuth salt, caustic alkali, and soluble silicate.

8. A dry reagent for testing urine for sugar containing an anhydrous bismuth salt, caustic alkali and a magnesium compound selected from the group consisting of the oxide and the carbonate, soluble silicate, and a stabilizer being a lead compound neutral in color.

9. A dry reagent of the class stated adapted to give the test on drop contact comprising a mixture containing a bismuth compound and caustic alkali, a magnesium compound and a lead compound, and soluble silicate in relatively large amount.

10. A dry reagent of the class adapted to give the sugar test on drop contact comprising a mixture containing an anhydrous bismuth salt and caustic alkali, a stabilizer for the mixture, and a soluble silicate neutral in color and having capillary structure, whereby the moisture rapidly penetrates the mass permeating its particles and a sharply defined test indication results.

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