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(54) STABLE IMMUNOLOGICAL REAGENTS

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do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to stable immunological reagents. More particularly, the invention is concerned with stable, water-dispersible freeze-dried powders containing a water-soluble dispersing agent formed from aqueous suspensions of latex particles having coupled thereto serologically determinant materials, as herein defined, and with a process for the manufacture of such powders.

Latex particles coupled to serologically determinant materials such as antigens and antibodies are known and are generally in the form of aqueous suspensions. The coupled latex particles are used in a variety of antigen-antibody test systems for demonstrating the presence of the corresponding antibody or antigen thus aiding in the diagnosis of medical conditions. The aqueous suspensions are satisfactory for use when fresh, but upon prolonged storge the suspensions are subject to attack by microorganisms and the spontaneous breakdown of the serologically determinant material. Furthermore, changes in the composition of the suspension due to evaporation of water can occur. The evaporation changes the ionic strength of the suspension, the reactivity in antigen-antibody systems being altered in many cases. There is thus a need for a stable latex-coupled serological determinant (e.g. antigens or antibodies) which remains stable upon prolonged storage.

This invention provides a stable, water-dispersible preparation of latex coupled to serological determinants as herein defined, in the form of a freeze-dried powder containing a water-soluble dispersing agent.

More particularly, the present invention provides a stable, water-dispersible freeze-dried preparation containing latex particles having chemically coupled thereto a serologically determinant material, as herein defined, and a sufficient amount of a dispersing agent to provide, upon reconstitution of the latex preparation to form a suspension with aqueous medium, 5 g/l to 30 g/l of said dispersing agent based on the volume of suspension of latex.

Latex suspensions cannot be dried by conventional means such as evaporation, spray-drying, freeze-drying or vacuum-drying because the procedures cause the latex to polymerise and cross-link. Due to the polymerisation, the latexes are not capable of being reconstituted into a homogeneous suspension.

This invention provides a process for drying aqueous suspensions of latex coupled to serological determinants, as herein defined, which forms stable, water-dispersible powders and which does not suffer from the problems expected when drying latex suspensions.

It has been discovered that the addition to the latex suspensions of a substance, such as a water-soluble saccharide compound which is capable of physically coating the particles of the latex coupled with serological determinants, as herein defined, prevents the polymerisation of the latex. The additive functions as a dispersing agent and polymeristion inhibitor.

In order to be satisfactory for use in the process of this invention, the additive must be water-soluble, capable of coating the coupled latex particles, compatible

with the latex reagent and inert with respect to the immunoreaction of the reconstituted latex suspension.

Typical of the suitable additives are sugars such as lactose, dextrose and sucrose, preferably lactose.

The amount of additive used varies with the system to which it is added. However, it has been found that adding an aqueous solution which contains the additive in a concentration of about 3% to up to 20% by weight, depending on the volume of suspension, when added to the suspension in an amount to provide from 5 g/l to 30 g/l of additive based on the volume of suspension of the coupled latex, is sufficient. The preferred amount after addition is 30 g/l of additive.

Less than 5 g/l of additive based on the volume of latex suspension is not effective, and more than 30 g/l of additive based on the volume of latex suspension can adversely affect the diagnostic use of the reconstituted suspension, depending on the system involved.

The term "serologically determinant materials" or "serological determinants" as used in the present description and claims is equivalent to the term "immunologically active materials" and refers to those materials which can be determined or can be used to determine specific components in human and animal body fluids by using immunological principles. Typical serologically determinant materials, as herein defined, are isolated human and animal antibodies and antigens. Specific serologically determinant materials, as herein defined, which are widely used or detected in medical diagnostics are chorionic gonadotropin, gammaglobulin, immunoglobulin G, immunoglobulin A, immunoglobulin M and human serum albumin. In addition, the said term embraces that specific identification of microorganisms which can be effected by coupling microoganism-identifying materials to the latex particles.

This invention includes within its scope the use of all those serologically determinant materials, as herein defined, which can be chemically bound to a serologically inert latex particle.

The term "serologically inert latex" as used herein denotes a latex which acts only as a carrier for the serologically determinant materials, as herein defined, and which has no effect on the immunological reaction in question. Suitable latexes are those which are water-insoluble, have a particle size in the range of from 0.01 to 0.9 microns and have a specific gravity near that of water so that after coupling with the respective serologically determinant material the specific gravity of the particles is about 1.1. In addition, the particles must be capable of chemically coupling to a serologically determinant material, as herein defined, without affecting its immunological properties.

Suitable latexes are latexes of carboxylated styrene butadienes, carboxylated polystyrenes, carboxylated polystyrenes containing amino groups, acrylic acid polymers, methacrylic acid polymers, acrylonitrile butadiene styrenes, polyvinyl acetate acrylates, polyvinylpyridines, or vinyl chloride acrylates. Examples of latexes coupled with serologically determinant materials (as herein defined) to which this invention applies are those disclosed in United States Patent Specification No. 3,857,931.

The process of this invention is carried out by forming an aqueous suspension of the coupled latex particles containing a suspending agent, preferably lactose. It is advisable that the suspension should be at a pH of from about 7.5 to 8.5. This is so because at this pH range the latex particles will not agglomerate and the serologically materials will not be adversely affected. The pH is usually maintained by a buffer, preferably a PBS (phosphate buffered saline), Tris [tris(hydroxymethyl)aminoethane hydrochloride]-saline or MES [2-(m-morpholino)ethane sulphonic acid and alkali metal salt thereof]-saline buffer. The preferred amount of lactose used is about 3% by weight/volume. The suspension is then usually divided into small aliquots (e.g. about 1 ml) in separate containers and frozen by immersion in a cold bath, preferably one composed of carbon dioxide and acetone.

The containers are then placed into a lyophiliser and dried *in vacuo* at about −70°C for about 48—72 hours, in typical cases. The resulting product is a porous powdery material which is about the same colour as the starting material. The lyophilised product contains about sufficient coupled latex to provide 7.5 mg in reconstituted 0.3 ml volume and sufficient lactose to provide 9 mg per 0.3 ml volume in the reconstituted product.

The powder can be reconstituted to the desired concentration by the addition of the correct amount of distilled water. Thus, if 1 ml aliquots are lyophilised, then

1 ml of water is added for each aliquot and stirred. This ensures reconstitution to form a suspension indistinguishable from the original. By this means, the original ionic strength of the coupled latex particles is restored, thus ensuring the desired immunological properties.

The following Examples illustrate the invention:

Example 1

Coupled latex antigen suspended in 0.01-*M* PBS (phosphate buffered saline), pH 8.0 containing 30 g/l lactose, is dispensed into ten 75 mm disposable culture tubes with rubber stoppers in a partially open position. The suspension is lyophilised in the tubes and dried *in vacuo* at –70°C for 48-72 hours. A porous powdery material is obtained.

Example 2

The powder prepared as described in Example 1 is reconstituted by adding, with vigorous mixing, sufficient distilled water to each test tube to replace the original volume prior to lyophilisation.

Example 3

Latex particles are washed in distilled water five times by centrifugation and then diluted 1—10 by volume in distilled water. 100 mg of extracted antigen from Group A streptococci is placed into a 15 ml polypropylene tube and 4 ml of distilled water are added to solubilise the antigen. 4 ml of the washed latex are added and the two components mixed on a Vortex mixer. 4 ml of 1% CDI [1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulphonate], freshly prepared in distilled water, are added and the components mixed. A magnetic stirring bar is added to the tube and the contents stirred. The coupling reaction is allowed to proceed for 2 hours at 22°—26°C. At the end of 2 hours, the latex-coupled antigen is centrifuged at 15,000 revolutions per minute for 30 minutes. The resulting supernate is discarded and the latex-coupled antigen is washed three times by centrifugation with 12 ml portions of 0.01-*M* PBS, pH 8.0. After the last wash, the latex-coupled antigen is re-suspended in 8 ml of 0.01-*M* PBS, pH 8.0 containing 30 g/l lactose. The resulting suspension is lyophilised as described in Example 1 and reconstituted as described in Example 2 to yield a stable product which has, upon reconstitution, reactivity identical to non-lyophilised controls which contain no added saccharide.

In a similar manner, HCG, immunoglobulin G, A and M and gammaglobulin are coupled to latex, lyophilised and reconstituted to yield stable products which have, upon reconstitution, reactivity identical to non-lyophilised controls which contain no added saccharide.

Latex control samples prepared as described in this Example and, to which no saccharide was added and which were lyophilised could not be resuspended.

The latex coupled to serological determinants is stable for about 2—3 weeks when stored at 4°C in 0.01-*M* phosphate buffered saline at pH 8.0. When lyophilised as described in Example 1 from a suspension containing 5 g/l to 30 g/l of lactose and stored at 4°C, the stability was increased to at least 36 weeks. The results are compiled in the following Tables. Table 1 shows the results of the inhibition reaction of latex-coupled antigen lyophilised in 0.01-*M* phosphate buffered saline (pH 8.0) containing 3%, 2%, 1% or 0.5% of lactose and reconstituted in water.

TABLE 1

Min.	Well A[1] Lactose Concentration (g/l)				Well B[2] Lactose Concentration (g/l)				Well C[3] Lactose Concentration (g/l)			
	30	20	10	5[4]	30	20	10	5	30	20	10	5
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	2+	1+	2+	2+	0	0	0	0
3	0	0	0	0	3+	2+	3+	2+	0	0	0	0
4	0	0	0	0	4+	3+	3+	3+	0	0	0	0
5	0	0	0	0	4+	4+	3+	3+	0	0	0	0

[1] – Well A contains whole streptococcal cells, dilutes antiserum and latex antigen and measures the reactivity of the test system.

[2] – Well B contains saline, diluted antiserum and latex antigen and is the control.

[3] – Well C contains whole streptococcal cells, saline and latex antigen and measures the autoagglutination of the latex.

[4] – When lactose was used at a concentration of less than 5 g/l, autoagglutination of the latex particles occurred upon reconstitution of the lyophilised antigen in water.

Table 2 shows the results of the inhibition reaction using latex-coupled antigen prepared in 0.01-*M* phosphate buffered saline (pH 8.0) and stored at 4°C or latex-coupled antigen lyophilised in 0.01-*M* phosphate buffered saline (pH 8.0) containing 30 g/l of lactose and used immediately after reconstitution in water.

TABLE 2

Min.	Latex antigen in 0.01–*M* PBS[1]					
	Week 1			Week 3–4		
	Well A[2]	Well B[3]	Well C[4]	Well A	Well B	Well C
1	0	0	0	0	0	0
2	0	1+	0	0	0	0
3	0	2+	0	0	0	0
4	0	3+	0	0	0	0
5	0	3+	0	0	0	0
Latex antigen lypholised in 0.01–*M* PBS containing 30 g/l of lactose						
Min.	Week 1			Week 36		
	Well A	Well B	Well C	Well A	Well B	Well C
1	0	1+	0	0	1+	0
2	0	2+	0	0	2+	0
3	0	3+	0	0	3+	0
4	0	4+	0	0	4+	0
5	0	4+	0	0	4+	0

[1] – Antigen stored at 4°C and subsequently used in the inhibition test.

[2] – Well A contains whole streptotoccal cells, diluted antiserum and latex antigen and measures the reactivity of the test system.

[3] – Well B contains saline, diluted antiserum and latex antigen and is the control.

[4] – Well C contains whole streptococcal cellsm saline and latex antigen and measures the autoagglutination of the latex.

[5] – Lyophilised antigen stored at 4°C, reconstituted in water and used immediately in the inhibition test.

In each Table 1 and Table 2
0 = no agglutination
1—4 = varying degrees of agglutination

If there is instability in the control (Well B) or interfering reactions there is no agglutination. If the test system (Well A) is stable (i.e. specifically in the presence of group A beta hemolytic streptococci) there is no agglutination. If there is instability in the latex system with no antiserum (Well C) there will be agglutination.

As shown in Table 1 and Table 2, the control retained its stability for up to 36 weeks when using the method of this invention. However, the control was not stable after the third week when not following the method of this invention.

WHAT WE CLAIM IS:—

1. A stable, water-dispersible freeze-dried preparation containing latex particles having chemically coupled thereto a serological determinant material, as herein defined, and a sufficient amount of a dispersing agent to provide, upon reconstitution of the latex preparation to form a suspension with aqueous medium, 5 g/l to 30 g/l of said dispersing agent based on the volume of suspension of latex.

2. A preparation according to claim 1, wherein the dispersing agent is a water-soluble saccharide.

3. A preparation according to claim 2, wherein the water-soluble saccharide is lactose.

4. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is Group A streptococcal antigen.

5. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is immunoglobulin G.

6. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is immunoglobulin M.

7. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is immunoglobulin A.

8. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is gammaglobulin.

9. A preparation according to any one of claims 1 to 3 inclusive, wherein the said serologically determinant material is human chorionic gonadotropin.

10. A preparation according to any one of claims 1 to 9 inclusive in the form of a powder.

11. A process for the manufacture of a stable, water-dispersible freeze-dried preparation, which process comprises adding to an aqueous suspension of latex particles chemically coupled to a serologically determinant material, as herein defined, a sufficient amount of an aqueous solution of a dispersing agent to provide 5 g/l to 30 g/l of said dispersing agent based on the volume of suspension of latex and lyophilising the mixture.

12. A process according to claim 11, wherein the dispersing agent is a water-soluble saccharide.

13. A process according to claim 11, wherein the water-soluble saccharide is lactose.

14. A process according to any one of claims 11 to 13 inclusive wherein the said serologically determinant material is Group A streptococcal antigen.

15. A process according to any one of claims 11 to 13 inclusive, wherein the said serologically determinant material is immunoglobulin G.

16. A process according to any one of claims 11 to 13 inclusive, wherein the said serologically determinant material is immunoglobulin M.

17. A process according to any one of claims 11 to 13 inclusive, wherein the said serologically determinant material is immunoglobulin A.

18. A process according to any one of claims 11 to 13 inclusive, wherein the said serologically determinant material is gammaglobulin.

19. A process according to any one of claims 11 to 13 inclusive, wherein the said serologically determinant material is human chorionic gonadotropin.

20. A process for the manufacture of a stable, water-dispersible freeze-dried preparation, substantially as hereinbefore described with reference to Example 1 or 3.

21. A stable, water-dispersible freeze-dried preparation, when manufactured by the process claimed in any one of claims 11 to 20 inclusive.

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