United States Patent [19]

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[11] **3,863,004**

[45] **Jan. 28, 1975**

[54] **DENATURED MACROPROTEIN WITH DIVALENT TIN FOR TAGGING WITH TECHNETIUM-99M AND METHOD OF PREPARATION**

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[22] Filed: **Mar. 20, 1972**

[21] Appl. No.: **236,159**

[52] **U.S. Cl.** ..... **424/1**, 252/301.1 R, 250/303

[51] **Int. Cl.** **A61k 27/04**

[58] **Field of Search** ..... 424/1; 252/301.1 R; 250/303

[56] **References Cited**

UNITED STATES PATENTS

3,663,686	5/1972	Grotenhuis et al.	424/1
3,663,687	5/1972	Evans	424/1
3,725,295	4/1973	Eckelman et al.	252/301.1 R

OTHER PUBLICATIONS

Lin et al., Journal of Nuclear Medicine, Vol. 12, No. 5 (1971) pp. 204–211.

Eckelman et al., Journal of Nuclear Medicine, Vol. 12, No. 11 (1971) pp. 707–710.

Morcellet, Use of Tin-Tc 99m-Nuclear Sci. Abs. Vol 24, No 4., p. 617, Feb. 28, 1970, Item No. 6078.

Charamza, Chemical Abstracts, Vol. 72, 1970 p.86, Item No. 87066.

De Paoli, Nuclear Sci. Abs., Vol. 20, No. 23 Dec. 15, 1966, Item No. 43390 "Albumin Macroaggregates etc."

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[57] **ABSTRACT**

An improved composition suitable for tagging with technetium-99m and use in lung scanning procedures consists essentially of an injectable suspension in buffer solution of particles of a denatured macroprotein having divalent tin bound thereto. The macroprotein has a molecular weight of at least about 20,000. A method for preparing the injectable suspension by treating macroaggregates or microspheres of a denatured macroprotein with a solution containing divalent tin ions is also provided.

14 Claims, No Drawings

DENATURED MACROPROTEIN WITH DIVALENT TIN FOR TAGGING WITH TECHNETIUM-99M AND METHOD OF PREPARATION

BACKGROUND OF THE INVENTION

This invention relates to the field of radiopharmaceutical diagnostic testing and more particularly to an injectable suspension of solid particulate denatured macroprotein suitable for tagging with technetium-99m and use in lung scanning procedures.

Macroaggregated macroproteins have found use as carriers for radionuclides useful in diagnostic radioscanning techniques. In particular, such proteins, for example human serum albumin labeled or tagged with a radionuclide, have been used in the diagnosis of pulmonary circulatory defects. The distribution in the lungs of radioactively labeled protein macroaggregates, as determined by isotope radioscanning, indicates the presence or absence of certain pathological conditions.

To be useful in lung scanning, radioactive macroprotein particles must be labeled with a gamma emitting radionuclide and be within a prescribed size range. The labeled particles must also be non-toxic and biodegradable, i.e., physically or chemically removable from the lungs in a relatively short period.

Macroaggregated human serum albumin tagged with iodine-131 has become widely used for perfusion lung scanning. While the biological properties of this scanning agent are excellent, an important objection to its use arises from the decay scheme of iodine-131. In the course of decay, iodine-131 emits a beta particle, its gamma rays are difficult to collimate effectively, and it has an 8-day physical half-life. As a result of these characteristics, a patient receiving an injection of iodine-131-tagged albumin is exposed to a high radiation dose, not only to the lungs but to other organs such as the thyroid gland as well.

A radionuclide which may advantageously be substituted for iodine-131 in lung scanning is technetium-99m which emits only gamma rays which can be easily collimated, and has a physical half-life of only 6 hours. Chiefly on the basis of favorable dosimetry profiles, a host of technetium-99m labeled particles have been prepared and used in lung scanning. A comprehensive review of the present state of knowledge in this field was published by G. V. Taplin and N. S. MacDonald in *Seminars in Nuclear Medicine*, Vol. 1, No. 2 (April), 1971, pages 132–52.

The safety, convenience and effectiveness of the lung scanning techniques employing technetium-99m have heretofore been limited, however, by problems associated with the preparation of technetium-tagged albumin. To prepare serum albumin for use in lung scanning, it must be both denatured and precipitated in the form of macroaggregates or microspheres of a particular range of particle size. To prepare the albumin in such form, a solution of albumin is typically heated to denature the protein after which the solution is cooled and the albumin precipitated by adjusting the pH to the isoelectric point (approximately pH 4.9). Particles of the desired size are obtained by suitable control of both pH and temperature. Alternatively, an aqueous solution of albumin can be dispersed in hot, water-immiscible oil to precipitate the albumin in the form of microspheres. Of the several known methods for tagging protein particles with technetium-99m, the most common has involved first tagging a solution of albumin with technetium and then precipitating to form the solid albumin in particles of the desired size. Where this method is utilized, however, an immediate microscopic examination of the particles must be made to assure their proper size, and purification is required to remove unreacted technetium from the suspension. Moreover, because of the short half-life of technetium-99m, sterility tests cannot be completed on the denatured and precipitated protein before it is used in human patients.

In an alternative method for preparing technetium-99m tagged macroaggregates, a technetium sulfur colloid is formed in the presence of denatured albumin microspheres causing the spheres to become tagged with the technetium. However, labeling is not very efficient by this method, and several washes of the protein particles are required to remove the unbound technetium. Also, the tagged microspheres produced by this method are prone to stick together and it becomes necessary to disperse the resulting agglomerates by an appropriate technique such as ultrasonic treatment, immediately prior to injection into a patient. Furthermore, the bond between the technetium and the microspheres is somewhat labile, and if the suspension is allowed to stand unused for as little as an hour or so after preparation, both the purification and ultrasonic treatment must be repeated before injection.

As a consequence of the various disadvantages outlined above, technetium-tagged albumin has not been found generally useful as a lung scanning agent, despite its desirable radiation properties.

SUMMARY OF THE INVENTION

Among the objects of the present invention may be noted the provision of an improved composition which may be readily and efficiently tagged with technetium-99m to produce an injectable suspension for use in lung scanning procedures; the provision of such a composition which can be stored for substantial periods of time without degradation, be tagged with technetium-99m and then used for injection without particle size examination, ultrasonic treatment or purification; and the provision of processes for preparing the composition. Other objects and features will be in part apparent and in part pointed out hereinafter.

In essence, therefore, the present invention is directed to a composition suitable for tagging with technetium-99m and use in lung scanning procedures. The composition consists essentially of an injectable suspension in a buffer solution of particles of a denatured macroprotein having divalent tin bound thereto, the macroprotein having a molecular weight of at least about 20,000. The invention is further directed to a process for preparing the composition. In this process, particles of a denatured macroprotein are contacted with a solution containing a water-soluble salt of divalent tin causing divalent tin to be bound to the macroprotein. The macroprotein having divalent tin bound thereto is then washed and suspended in buffer solution.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It has now been discovered that an injectable suspension of a denatured and precipitated macroprotein having divalent tin bound thereto may be prepared and stored without degradation for substantial periods prior to use. Even more significantly, it has been found that

the macroprotein of the suspension may be quickly and easily tagged with technetium-99m and that the resulting suspension of the tagged protein may be safely and effectively used in lung scanning techniques without particle size determination, ultrasonic treatment or purification for the removal of unbound technetium. Thus, the relatively cumbersome and time-consuming techniques previously required are avoided and both the cost of preparing a suitable suspension for use in lung scanning procedures and the risks associated with its use are minimized.

The macroprotein constituent of the composition of the invention is preferably denatured human serum albumin, a protein which is particularly suitable for use in lung scanning. It will be understood, however, that any denatured macroprotein having a molecular weight of at least about 20,000 may have divalent tin bound thereto in accordance with the invention to render it more susceptible to labeling with a radioactive isotope of technetium.

The particles of the protein constituent of the composition of the invention are characterized as "macroaggregates". Macroaggregates are considered to be particles of irregular form having an average diameter of between about 5 and about 80 microns. Where the protein particles are intended for use as lung scanning agents, the mean size of the particles is preferably between about 15 and about 30 microns.

The improved susceptibility of the suspended protein macroaggregates to tagging with technetium-99m relates to the divalent tin which is bound to the protein. When a suspension of serum albumin treated with divalent tin in accordance with the invention is subsequently treated with a solution containing heptavalent technetium-99m, for example a solution of sodium pertechnetate, upwards of 90% of the technetium is rapidly and firmly bound to the serum protein.

In the practice of the invention, human serum albumin or other appropriate macroprotein is first denatured and then precipitated to form particles of the desired size. Various methods are known to the art for producing macroaggregates or microspheres of denatured protein. While any of these methods may be successfully used, excellent results have been obtained using a method wherein a dilute aqueous solution of human serum albumin is first heated at a temperature of about 70°–100°C., preferably about 85°C., to denature the protein, then cooled to a temperature on the order of 18°–22°C., preferably about 20°C. The albumin is then precipitated by slowly adding a dilute acid, approximately 0.1N, until the pH is about 4.8–5.2, i.e., the isoelectric pH of the protein being about 4.9. Hydrochloric acid is preferred for use in adjusting the pH to precipitate the denatured protein. However, other mineral acids or acetic acid may also be employed. The particle size of the macroaggregates thus precipitated is typically in the range of 5–80 microns with a mean particle size of 15–30 microns. The integrity of the precipitated aggregates is further improved if the suspension is reheated for a short period of time following precipitation. Preferably the reheating temperature is of approximately the same order as the denaturing temperature.

After reheating, the macroaggregated albumin particles are separated from the suspension, washed and resuspended in a fresh buffer solution having essentially the same pH as the isoelectric pH of the protein (i.e., pH 4.9). A convenient buffer solution is an acetate buffer having a pH of 4.9 (0.052N sodium acetate and 0.02N hydrochloric acid).

Binding of the protein aggregates with divalent tin is effected by adding to the protein suspension an aqueous solution containing stannous ions. Contact of the macroaggregates with stannous ions causes divalent tin to become bound to the protein. For this purpose, stannous chloride is preferred because of its solubility and greater stability, although other water-soluble stannous salts, such as stannous sulfate, may also be used. It is necessary, however, to employ a divalent tin solution since tetravalent tin ions are not effective.

Sufficient stannous ions are available for binding to the protein if the mixture of the protein suspension and stannous salt solution contains approximately 3 mg. of stannous ions per 100 mg. of macroaggregates. With such proportions, binding of divalent tin to the protein molecule occurs quickly at room temperature and appears to be substantially complete in about 15 minutes or so. For optimum results, however, the mixture containing stannous ions and protein should be allowed to stand for a longer time, preferably about 24 hours. After the treatment period is complete, the aggregates are separated from the liquid phase, washed with and resuspended in fresh buffer solution. After resuspension, the macroaggregates may be stored for a substantial period at 4°C.

In an alternative embodiment of the invention, the precipitation and tin-binding steps of the process are combined into a single step. Following this alternative, the stannous salt is dissolved in the dilute acid used to precipitate the heat-denatured protein thus eliminating one step of the above-described process. Whichever procedure is followed, the same end result is realized, i.e., the preparation of a suspension of precipitated denatured protein particles having divalent tin bound thereto.

The resulting suspensions have a shelf life of approximately 60 days when stored at 4°C. before such characteristics as particle integrity and susceptability to radionuclide tagging begin to deteriorate. If 60 days of storage are significantly exceeded, the tagging efficiency normally falls off to the extent that the product should be discarded.

The shelf life of the macroaggregate suspensions may be extended beyond 60 days by freezing the suspension and storing it in the frozen state. The shelf life of the frozen suspension has been found to extend to approximately 4 months or longer. Storage in the frozen state has not generally been attractive, however, since the macroaggregate particles tend to clump together during subsequent thawing and form agglomerates which are too large for use in lung scanning procedures.

This problem is overcome by incorporating into the suspension between about 8% and about 16% by weight of an antiagglomerating agent such as propylene glycol, glycerin, gelatin or sucrose. The preferred antiagglomerating agent is propylene glycol. Agglomeration of the macroprotein particles is effectively prevented by inclusion of the anti-agglomerating agent in the suspension immediately prior to freezing.

To prevent bacterial growth in the suspension, the addition of a suitable preservative is also advisable. For this purpose, the addition of approximately 1% by weight of benzyl alcohol to the initial protein solutions or to the finished suspensions of the invention has been

found acceptable and effective. Other nontoxic bacteriostatic preservatives may be substituted for benzyl alcohol.

The tin-treated protein of the suspension is conveniently and easily tagged with technetium-99m by adding the sodium pertechnetate eluate from a technetium generator directly to the suspension. Generators suitable for preparation of the eluate are commercially available. For example, one such generator is described in Shumate U.S. Pat. No. 3,535,085, dated October 20, 1970. When the eluate and suspension are mixed, binding of the technetium to the protein occurs spontaneously and quickly with an efficiency of 95% or more. Preferably, about 30 minutes are allowed to optimize the specific activity of the tagged suspension. The mechanism of tagging is not clear but it involves oxidation of the divalent tin to the tetravalent state with concomitant reduction of the heptavalent technetium to a lower valency. Tetravalent tin remains attached to the protein. Within minutes of the addition of the technetium eluate thereto, the suspension of tagged albumin may be intravenously injected directly into animals or humans without any further treatment.

The effectiveness of lung scanning is optimized by using sufficient eluate to provide a tagged suspension having a specific activity of up to approximately 20 mci TcO_4/mg. of the macroaggregated albumin. Five minutes after injection, the lungs of a subject contain 90% or more of the injected technetium-99m. The presence or absence of a pathological condition is then determined by radioscanning the lungs of the patient and comparing the emission pattern thereof with a standard pattern.

The tagged denatured albumin is eliminated from the subject's lungs at a rate corresponding to a biological half life of approximately 5–10 hours.

Thus, the suspensions of the tin-treated albumin macroaggregates may be stored for substantial periods of time while still retaining their potency. Then, when desired, they may be tagged with technetium merely by adding a solution containing sodium pertechnetate for immediate use as a lung scanning agent without further treatment. The invention thus obviates the costly, time-consuming and difficult procedures which have heretofore been necessary.

The following examples illustrate the invention.

EXAMPLE 1

A solution containing fresh human serum albumin (500 mg.) in a 1% by weight aqueous solution of benzyl alcohol (45 cc.) was heated for 15 minutes at 85°C. with slow stirring to denature the protein. The solution was then transferred to a cold water bath, and when the temperature had reached 18°–22°C. 2 ml. of 0.085N hydrochloric acid solution was slowly added over a period of about 3 minutes with rapid stirring. The pH of the resulting suspension of precipitated albumin was 4.8 to 5.2. The suspension was then reheated to 83°C. for 5 minutes with slow stirring, causing the precipitated albumin aggregates to become more compact and less subject to fragmentation. The particle size analysis of the precipitated macroaggregates indicated the size range to be 5–80 microns, with a mean of 15–30 microns. After reheating, the macroaggregates were collected by centrifugation and resuspended in 30 ml. of an acetate buffer solution having a pH of 4.8 (0.052N sodium acetate and 0.02N hydrochloric acid).

EXAMPLE 2

To the suspension produced in Example 1 was added 0.6 ml. of a 1N HCl solution containing 50 mg. of stannous chloride per ml. This is equivalent to approximately 3 mg. of divalent tin per 100 mg. of albumin. The pH of the resulting mixture was 4.0–4.1. Two ml. of a 0.4M sodium acetate solution was added, and the mixture allowed to incubate for 24 hours. After the incubation period was complete, the protein macroaggregates were washed three times by centrifugation, with resuspension in 30 ml. of fresh acetate buffer following each washing. The treated particles were finally resuspended in 50 ml. of pH 4.8 acetate buffer containing 1% by weight benzyl alcohol.

The resulting suspensions may be stored at 4°C. or frozen as previously described. If they are to be stored at 4°C., the suspensions are diluted 1:5 with acetate buffer (pH 4.8) containing 1% benzyl alcohol. If they are to be frozen the suspensions are diluted 1:5 with acetate buffer (pH 4.8) containing 1% benzyl alcohol and 8–16% propylene glycol, for example.

In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

As various changes could be made in the above methods and products without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

What is claimed is:

1. A composition suitable for rapid tagging with technetium-99m and use in lung scanning procedures consisting essentially of an injectable suspension in a buffer solution of particles of denatured macroprotein having divalent tin bound thereto, said macroprotein having a molecular weight of at least about 20,000.

2. A composition as set forth in claim **1** wherein the pH of said buffer solution is approximately the isoelectric pH of said denatured macroprotein.

3. A composition as set forth in claim **1** wherein said macroprotein comprises particles of macroaggregated human serum albumin.

4. A composition as set forth in claim **1** wherein the size of the particles is substantially between about 5 and about 80 microns.

5. A composition as set forth in claim **4** wherein the mean size of said particles is between about 15 and about 30 microns.

6. A composition as set forth in claim **1** including macroprotein particles in the form of microspheres.

7. A composition as set forth in claim **1** wherein the buffer solution contains between about 8 % and about 16 % by weight of an antiagglomerating agent selected from the group consisting of propylene glycol, sucrose, glycerin and gelatin.

8. A process for preparing a buffered injectable suspension of particles of denatured macroprotein suitable for rapid tagging with technetium-99m and use in lung scanning procedures comprising the steps of:

contacting particles of a denatured macroprotein with a solution containing a water-soluble salt of divalent tin causing divalent tin to be bound to said macroprotein;

washing the macroprotein having divalent tin bound thereto with a buffer solution; and

thereafter suspending in a buffer solution the macroprotein having divalent tin bound thereto.

9. A process as set forth in claim **8** wherein said macroprotein is human serum albumin.

10. A process as set forth in claim **8** wherein said solution containing divalent tin ions is a solution of stannous chloride.

11. A process as set forth in claim **10** wherein said solution contains approximately 3 mg. of divalent tin for each 100 mg. of macroprotein.

12. A process as set forth in claim **8** wherein said macroprotein is in the form of macroaggregates.

13. A process as set forth in claim **8** wherein said macroprotein is in the form of microspheres.

14. A process as set forth in claim **8** wherein said macroprotein is prepared by precipitating denatured macroprotein in an acid medium at a pH approximately the isoelectric pH of said protein.

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