

[54] TC-99M ALBUMIN AGGREGATES WITH
STANNOUS TIN AND DENATURED
ALBUMIN

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 164,456, July 20,
1971, abandoned.

[52] U.S. Cl. **424/1**, 23/230 B, 250/303

[51] Int. Cl. **A61k 27/04**, G21h 5/02

[58] Field of Search 424/1; 252/301.1 R;
23/230 B; 250/303

[56] **References Cited**

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[57] **ABSTRACT**

Disclosed herein are aggregatess comprising coprecip-
itated Tc-99m, ionic tin and denatured albumin. The
aggregates of this invention can be utilized in the im-
aging of lungs to determine the conditin of pulmonary
vessels.

9 Claims, No Drawings

TC-99M ALBUMIN AGGREGATES WITH STANNOUS TIN AND DENATURED ALBUMIN

This application is a continuation-in-part of application Ser. No. 164,456 filed July 20, 1971, now abandoned.

BACKGROUND OF INVENTION

Human serum albumin products tagged with radioactive iodine have been used to scan various areas of the body. In addition, Tc-99m in recent years has gained wide acceptance as a tagging moiety. Various procedures have been adopted or proposed by which albumin can be tagged with Tc-99m rather than utilizing radioactive iodine.

The value of Tc-99m for clinical scintiscanning stems both from its physical and from its chemical properties. Its short half-life, of 6 hr., coupled with an absence of primary β -emission, result in very low radiation doses to tissues. The high activities that can therefore be administered shorten the time required for a scan and enable several "views" of an organ to be examined. The low energy of its γ -emission, 140 KeV enables a picture to be obtained with a focusing collimator, of virtually a surface layer a few cm in thickness. This due partly to improved resolution, and partly to reduction of the contribution of radiation from deeper structures by self-absorption.

As mentioned above, technetium can be prepared in a variety of chemical forms and can be complexed with proteins. The use of Tc-99m complexed with macroaggregated human albumin for lung scanning has been described.

In an article by Gwyther, M. M. and Field, E. O.; Aggregated Tc^{99m}-Labelled Albumin for Lung Scintiscanning, *Int. J. Appl. Rad. and Isotopes*, 17:485-486, 1966, there is described a procedure for labelling and aggregating albumin by utilization of increased concentration of ferric chloride and ascorbic acid.

A different approach to the problem is described by DePaoli, T., Hager, A., Nicolini, J. O.; Albumin Macroaggregates Labelled with Tc^{99m}, *Inter. J. Appl. Rad. and Isotopes*, 17:551-554, 1966.

In this procedure Tc-99mO₄ is reduced with hydrochloric acid and ammonium thiocyanate. A protein precipitate, which retained more than 95 percent of the Tc-99m, was formed when the solution of reduced technetium was added to the human serum albumin.

A more complete detailed description of macroaggregates is set forth in an article by Bowen, B. M. and Wood, D. E.; Preparation of Technetium-99m Human Serum Albumin Macroaggregates, *Am. J. of Hospital Pharm.*, 26:529-534, 1969.

In this article the criteria for an acceptable macroaggregate product are set forth as follows:

1. It is sterile and pyrogen-free;
2. Less than 5 percent of the total radioactivity is not organically bound;
3. Less than 10 percent of the particles are less than 5 microns in the greatest diameter, and no particle is greater than 100 microns. The mean distribution must fall between 10 and 50 microns;
4. Specific activity is such that the required dose of radioactivity can be carried on less than 1.5 mg. of albumin.

All of these procedures have the major disadvantage of requiring skilled personnel and special equipment to

carry out the tagging procedures. There have been attempts to simplify these procedures; however, each procedure developed still requires numerous mixing and heating steps.

SUMMARY OF INVENTION

This invention relates to the tagging of human serum albumin with the radioactive isotope, Tc-99m, without carrying on numerous reactions and heating procedures. It has been discovered that aggregates of albumin and stannous chloride can be treated with Tc-99m, and the resulting suspension formed can be injected intravenously into the body.

DESCRIPTION OF INVENTION

The aggregates formed by the present invention contains what is known in the art as macroaggregated human serum albumin.

The process of this invention comprises adding stannous halide (e.g., stannous chloride, stannous iodide, stannous bromide, etc.) to an acidified solution of human serum albumin, adjusting the pH to the isoelectric point of albumin to form macroaggregates, removing the macroaggregate supernatant thus formed, re-suspending the macroaggregates formed in a solution suitable for injection to a specific concentration which may or may not then be dehydrated and adding Tc-99m solution to form radioactive tagged macroaggregates.

The term macroaggregate as utilized in this description means a particle having a size of from about 5 to 100 microns with the preferred size being from about 30 to 50 microns.

The macroaggregates of this invention are formed utilizing human serum albumin which is denatured.

The denatured albumin of this invention is known in the art as first stage denatured albumin and can be prepared by any known means. One manner in which the albumin can be prepared is by heating normal serum albumin (NSA) followed by making it alkaline. Human normal serum albumin is to be utilized when the test is to be performed in humans and appropriate animal serum is utilized when the test is performed on cows, dogs, rats, and so forth. The NSA is heated to a temperature of from between about 55 to 95 degrees Centigrade, but more preferably from between about 75 to 85 degrees Centigrade for from 15 to 60 minutes, depending on the quantity of albumin utilized. The larger quantities of albumin requiring longer periods of time than smaller quantities. Utilizing an inorganic acid such as sulfuric acid, hydrochloric acid, nitric acid, and the like, an organic base such as sodium acetate, acetic acid buffer solution, an inorganic base such as alkali metal hydroxide (e.g., sodium hydroxide), or alkali metal bicarbonate (e.g., sodium bicarbonate) the pH of the albumin is adjusted to between about pH 4.5 to 6, but preferably to pH 5.3. This causes denatured albumin to precipitate. It should be noted that the albumin may be denatured by adjusting the pH to about 8, wherein an acid is used to adjust the pH to about 4.5 to 6, or the albumin may be denatured by adjusting the pH to about 2, wherein a base is employed to reach a pH of about 4.5 to 6. The pH change is generally accompanied by warming to insure adequate denaturation.

The preferred manner in preparing the albumin of this invention is by utilizing commercially available

human serum albumin having preservatives and anti-foam agents therein. This material is adjusted to a pH of from about 1.00 to 3.0, but preferably to about 1.50 to about 2.00 utilizing a strong inorganic acid, e.g., sulfuric acid, hydrochloric acid, etc. Stannous halide dissolved in a chloride acid solution is then added to the reaction mixture. The ratio of stannous ions to albumin should be from about 1:2 to 1:20. The most outstanding results being achieved when the ratio of stannous ions to albumin is about 1:5.

After the denatured HSA is prepared the requisite amount of stannous halide in an acid medium such as hydrochloric acid is added. The pH of this solution should be about 1.0 to 2.0 but preferably about 1.1 to 1.3.

The solution is then adjusted to the isoelectric point of denatured albumin which is about pH 5.3 but could reasonably fall within 4.8 to 5.8. Particulate matter is thus formed which have become known in the art as macroaggregates. To assure that these macroaggregates become firm, heat is applied to the solution for a period of from 10 to 45 minutes with from 10 to 20 minutes being the preferred period of time. The temperature to be applied is from about 75° to 85°C with the preferred range being from 78° to 81°C.

The macroaggregates thus formed are then washed with distilled water, saline solution or a reducing agent such as a sulfonic acid salt solution. The particles are then resuspended in water for injection, saline, or a solution containing a suitable reducing agent such as dextrose, salt of sulfonic acid, and so forth. The macroaggregates can also be dehydrated if desired, since this appears to enhance the stability of the stannous ion. The preferred method of dehydration is lyophilization although other standard methods may be employed.

Although the description herein has the use of albumin to form the macroaggregates of this invention, other proteins such as globulin may be utilized. Thus, alpha, beta or gamma globulins can be utilized with fibrinogen being one of the most preferred globulins.

The procedures to this point are conducted in a manner as to render the particles sterile and non-pyrogenic. They are thus in a form suitable for injection. Such dosage units are prepared so that a single intravenous injection when administered to a mammal of about 70 kg of body weight will deliver about 1 to 5 millicuries of Technetium-99m, preferably about 3 millicuries.

In the practice of this invention a vial of preformed macroaggregates is delivered to a technician. All that need be done by the technician is to add the required amount of Tc-99m from a generator as disclosed in U.S. Pat. No. 3,369,121.

To the solution is added the desired amount of Technetium-99m in an aqueous solution, preferably a saline solution, as predetermined by the practitioner, it being understood that the amount of radioactivity desired is the governing factor in how much Technetium-99m is to be utilized. Measurement of chemical quantities of Technetium is impractical as it is present in varying amounts depending on the activity of the generator and when it was last eluted. The reaction proceeds equally well by reversing the addition of reagents in solution to Technetium-99m. A stannous salt of a mineral acid is generally utilized in the practice of this invention, preferably a halide, such as stannous chloride, stannous bromide, stannous iodide, etc.

The following examples are illustrative of this invention. All temperatures are in degrees centigrade unless otherwise stated:

EXAMPLE 1

Preparation of Albumin Macroaggregates

Reagents	Amount
Human Serum Albumin (HSA) 25%	0.4 ml (100 mg HSA)
Benzyl Alcohol	0.9 ml
Propylene Glycol	2.0 ml
Antifoam AF	ca. 2 drops
Water for Injection	75 ml
0.1N HCl q.s. ad pH 1.75	1.1 ml
SnCl ₂ ·2H ₂ O	100 mg
0.5M Acetate Buffer q.s. ad pH 5.1	20 ml

The human serum albumin, benzyl alcohol, propylene glycol, antifoam, and water for injection are combined and mixed. After the mixture is adjusted to 1.75 with the 0.1N HCl, stannous chloride is added and mixed. The 0.5M acetate buffer is added with mixing to form macroaggregates and the mixture heated in a 80°C (± 15°C.) water bath for 40 minutes while constantly being stirred. The mixture is centrifuged and the supernatant discarded. To further purify the macroaggregates, water for injection is mixed with macroaggregates, the mixture again centrifuged and the supernatant discarded. The denatured macroaggregated human serum albumin is suspended in 100 ml of water for injection and refrigerated (ca. 5°C).

EXAMPLES 2 AND 3

The reagents and procedural outline for making 2 preparations of albumin macroaggregates.

Each preparation is tested for radiochemical binding and the amount of the injected dose that located in the lungs and liver of rats. After the preparations are made, exactly 0.25 ml is injected into the external jugular vein of rats. Three rats are used per preparation. After a resident time of 15 minutes in the rats, they are sacrificed and the percent of the injected dose in the lungs and liver determined. To determine the radiochemical binding, a count rate on the remainder of each formulation is made. They are then centrifuged and a count rate taken on the decanted supernatants only. The percent radiochemical binding is determined as follows:

$$\% \text{ Binding} = \frac{\text{Total count rate} - \text{Supernatant count rate}}{\text{Total count rate}} \times 100$$

Table I

	Ex. 2	Ex. 3
Macroaggregated HSA Suspension (Ex. No. 1)	2 ml	1 ml
Potassium Biphthalate Buffer	1 ml	—
Tc-99m (from generator)	2 ml	1 ml
% Injected Dose in Rat Lungs	95.4*	99.6*
% Injected Dose in Rat Liver	2.1*	0.63*

*Average of three rats.

From these examples it is seen that aggregates of Ionic Tin - Tc-99m macroaggregate particles can be readily prepared and used as a lung scanning agent.

EXAMPLE 4

Preparation of Macroaggregates

Reagents	Amount
Human Serum Albumin (HSA) 25%	0.4 ml (100 mg HSA)
Water for Injection	50 ml
0.1N HCl q.s. ad	pH 2
2.5% SnCl ₂ ·2H ₂ O in 0.1N HCl	1 ml
1M Acetate Buffer q.s. ad	pH 5.1-5.2

The human serum albumin, water for injection, and 0.1N HCl are combined and mixed for 20 minutes at 79°C. (ca. 325 oscillations/min.). The 2.5% stannous chloride solution is added and the pH adjusted to 5.1-5.2 with the 1M acetate buffer solution (ca. 30 ml). The mixture is again heated with mixing for 20 minutes at 79°C. (ca. 325 oscillations/min). The mixture is centrifuged and the supernatant discarded. To wash the macroaggregates, water for injection is mixed with the macroaggregates, the mixture again centrifuged, and the supernatant discarded. This washing step is repeated then the macroaggregates suspended in 100 ml of water for injection and refrigerated.

EXAMPLE 5

Following the procedure of Examples 2 and 3 the percentage uptake is set forth in Table II:

Table II

Macroaggregated HSA Suspension (Ex. No. 2)	1 ml
Tc-99m (from generator)	1 ml
Water for Injection	8 ml
% Injected Dose in Rat Lungs	108*
% Injected Dose in Rat Liver	0.57*

*Average of three rats.

This table illustrates the binding ability of the ionic-tin macroaggregate.

EXAMPLE 6

Normal human serum albumin (.75 g) is added to a mixture of hydrochloric acid (.5 N) (20 ml) and water for injection (400 ml) and heated to 80°C for 30 minutes with agitation. To the cooled mixture, stannous chloride solution (1%) (7.0 ml) is added through a sterilizing filter followed by the immediate addition of an acetate buffer (1 m) (100 ml) [sodium acetate, anhydrous 8.5 g; glacial acetic acid 4.0 ml; water for injection q.s. to 100 ml]. The mixture is reheated to 80°C for

15 minutes with agitation after which the agitation is stopped and the aggregates allowed to settle. The aggregates are resuspended in water for injection after the volume needed to give the desired denatured albumin concentration of 0.75 mg/cc is determined based on a chemical analysis. Based on the calculated final volume, the amount of normal human serum albumin necessary to make the product a 0.5% solution with respect to this carrier is determined. Vials (preferably silicon) are filled and the contents lyophilized to dryness. Before the vials are sealed they are flooded with nitrogen.

At time of use a Tc-99m solution, depending on its source the radioactive concentration may range from .5 millicuries per ml to 200 millicuries per ml; generally, however one adjusts the solution so as to add 1 to 3 ml of the Tc-99m solution having a radioactive concentration of 1 to 10 millicuries per ml.

What is claimed is:

1. A macroaggregate of Technetium-99m, denatured albumin and stannous tin.

2. A macroaggregate in accordance with claim 1 being from about 5 to 100 microns in dimension.

3. A process for preparing macroaggregates of claim 1 which comprises complexing denatured albumin with a stannous salt, adjusting the pH of the reaction solution to from between about 4.8 to 5.8 to form macroaggregates and adding an aqueous Tc-99m solution thereto.

4. A process in accordance with claim 3 wherein the pH is adjusted with an inorganic base.

5. A process for preparing macroaggregates in accordance with claim 1 which comprises adding Technetium-99m to a reaction vial containing macroaggregates of denatured albumin and stannous tin.

6. The process of claim 3 wherein said macroaggregate solution is dehydrated prior to the addition of the aqueous Technetium-99m solution.

7. A method for scanning the lungs the pulmonary system which comprises injecting intravenously the macroaggregate of claim 1 in a pharmaceutically acceptable vehicle and scanning the lungs and pulmonary system.

8. A macroaggregate of Technetium-99m, denatured albumin or globulin, and stannous tin.

9. A method for scanning the lungs and pulmonary system which comprises injecting intravenously the macroaggregates of claim 8 in a pharmaceutically acceptable vehicle and scanning the lungs and pulmonary system.

* * * * *

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 3,872,226
DATED : March 18, 1975
INVENTOR(S) : Thomas A. Haney et al.

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

In the abstract page, paragraph entitled ABSTRACT,
"conditin" should read--condition--. Column 1, line 39,
"concentration" should read--concentrations--. Column 2,
line 45, "55 to 95 degrees" should read--55 and 95
degrees--. Column 4, line 26, "80°C" should read--80°C.--.

Column 6, line 39, "the pulmonary" should read--and
pulmonary--.

Signed and Sealed this

fifth Day of August 1975

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

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[SEAL]

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