LABELING ALBUMIN MICROSPHERES WITH ^{113m}In

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Albumin microspheres labeled with short-lived nuclides have recently been introduced as a useful radiopharmaceutical for lung scanning and for studies of the circulation such as the measurement of arteriovenous shunting (*1*). In addition, to improved uniformity in size and shape, another advantage of these radioactive particles is that they can be prepared in large batches and labeled immediately before use. This eliminates the variation in the distribution of particle size which has been a problem when different batches of particles are prepared.

The purpose of this paper is to describe a method of labeling albumin microspheres with ^{113m}In, a well-established (*2*) generator-produced radionuclide with a 1.73-hr half-life and 390-keV gamma-ray emission.

MATERIALS AND METHODS

Human serum albumin microspheres which contained iron hydroxide were obtained from The 3M Company. The various sizes of different batches of the microspheres ranged from 5 to 70 microns. Figure 1 shows the particle size distribution of several batches of microspheres. Figure 2 shows the relationship of microsphere diameter to the number of microspheres per milligram.

Ten-milligram portions of the microspheres were weighed and placed in clean, dry, dust-free vials. The presence of moisture must be avoided since it leads to clumping of the microspheres during the labeling procedure. The dry microspheres were autoclaved 10 min at 150 psi and 250°F and stored in the dark until use. All reagents were prepared with sterile, pyrogen-free water. A large supply of reagents was prepared at one time and placed in small vials that were autoclaved and stored until use.

Ionic ^{113m}In was eluted from a ^{113}Sn-^{113m}In generator (Union Carbide) with 0.05 *N* HCl and the volume adjusted to 5–10 ml with additional 0.05 *N* HCl. After adjusting the pH to 3 with 0.2 *M* $Na_2HPO_4 \cdot 7H_2O$ and adding 0.1 ml 5% Tween 80, the ^{113m}In solution was passed into the vials containing the microspheres through a Millipore Swinnex-13 unit containing a 0.45-micron filter to obtain a sterile solution. Filtration also assured the removal of particulate impurities in the ^{113m}In solution. Such impurities, which were rarely present, seemed to interfere with the labeling.

Received Nov. 18, 1968; original accepted Jan. 9, 1969.

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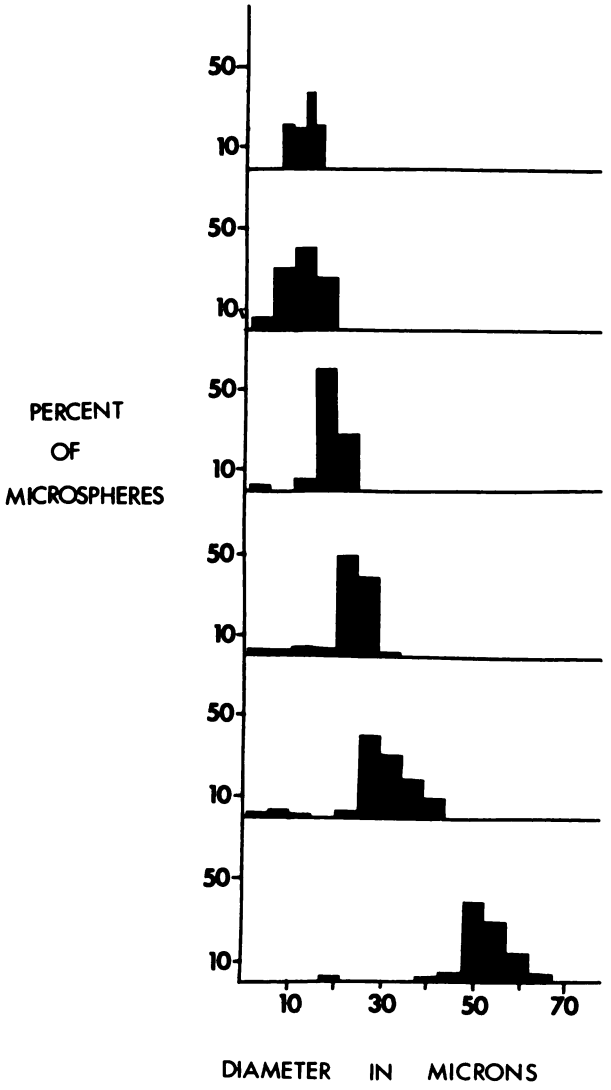

FIG. 1. Particle size distribution of various preparations of human serum albumin microspheres containing iron hydroxide.

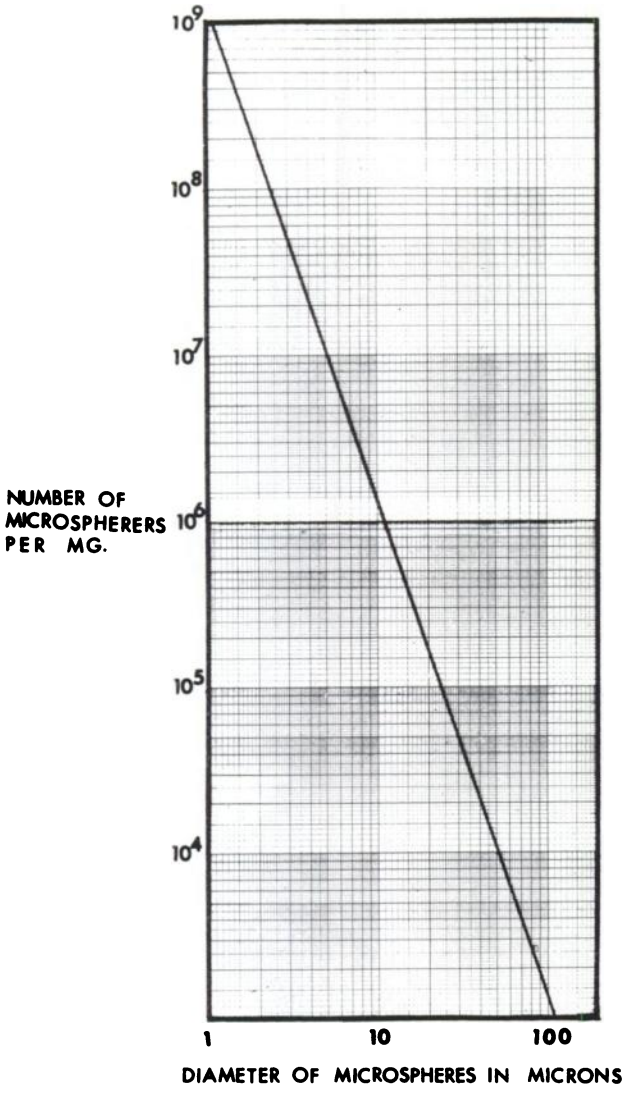

FIG. 2. Relationship of number of microspheres per unit weight as function of diameter.

The labeling procedure is outlined in Fig. 3. The vial containing the microspheres and the ionic indium was incubated for 30 min at 75°C. The residual free ^{113m}In was measured in the supernate. If the free ^{113m}In was greater than 5%, the supernate was removed and the microspheres washed with normal saline. Before injection they were resuspended in an appropriate volume of normal saline or 6% Dextran.

The microspheres were examined microscopically at various stages of the labeling procedure. Occasionally they appeared clumped. If this happened the vial was exposed to ultrasonification, which did not change the properties of the microspheres but did disperse them.

In developing the labeling procedure, the time and temperature of the incubation and the effect of pH were studied. The samples were incubated in a shaking water bath and aliquots removed at time intervals from 1 min to 5 hr. Experiments were performed at room temperature, 50°, 75° and 100°C. Solutions of various pH were obtained with phosphate buffers or phosphate buffers with added phosphoric acid or sodium hydroxide.

Two tests were used to determine the stability of the label. First, labeled microspheres were washed several times with saline and the activity in the supernate measured. Second, the biological stability of the labeled microspheres was tested by determining their distribution in white mice. The injections were made into a tail vein and the content of activity in the mice was measured immediately in a well scintillation counter. The mice were sacrificed 10 min to 5 hr later. The activity of the lungs, liver, tail and carcass was determined. The percent of the dose in each sample was determined after correction for the activity remaining in the tail. The lung-to-liver ratio was the criterion used to establish the stability of the preparation.

RESULTS

Factors that affected the efficiency of labeling the microspheres with ^{118m}In were: (1) the time of incubation; (2) the temperature of the water bath; and (3) the pH and concentration of the phosphate solution. When the microspheres were incubated at 75°C and the pH was 3, 94% labeling was achieved

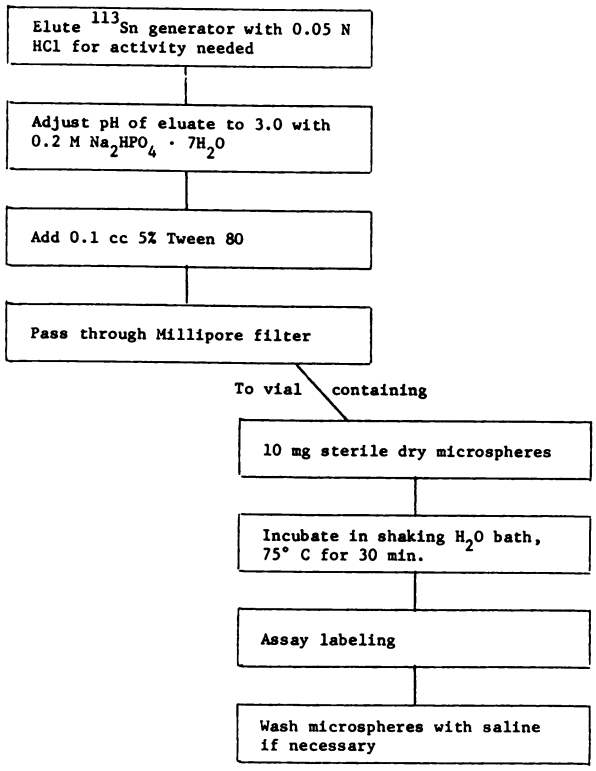

FIG. 3. Method for labeling human serum albumin microspheres containing iron hydroxide.

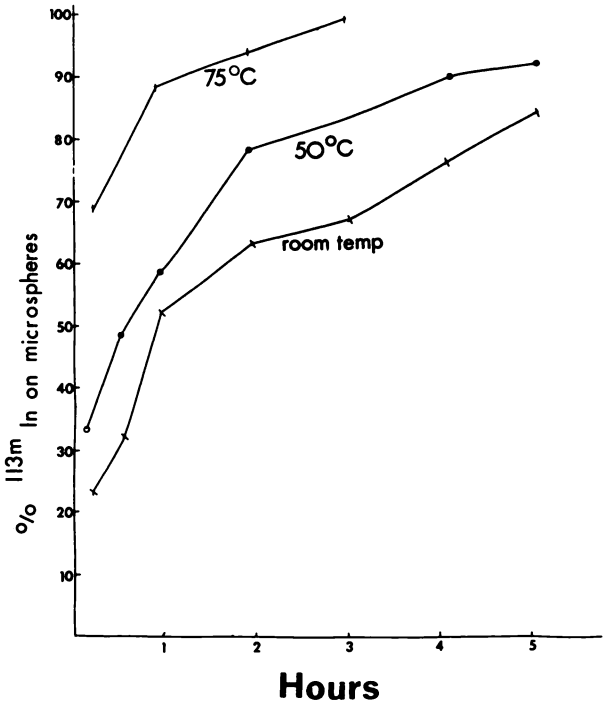

FIG. 4. Microspheres were incubated with ^{113m}In in phosphate buffer at pH 4.5 at various temperatures and labeling measured as function of time.

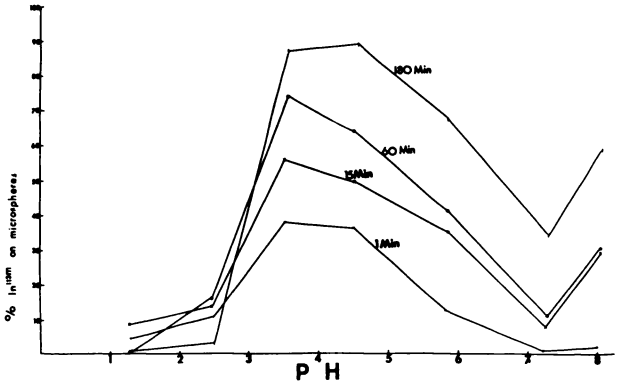

FIG. 5. Microspheres were incubated with ^{113m}In at 50°C in phosphate solutions varying in pH from 1.3 to 8 and labeling measured as function of time.

in 30 min. The incubation was limited to 30 min because of the 1.73-hr half-life of the ^{113m}In. The temperature was limited to 75°C because labeling carried out at higher temperatures sometimes led to breakdown of the microspheres. The optimum binding occurred between pH 3 and 4.5. The former (pH 3) was preferred because the microspheres were slightly basic, which caused a small rise in pH after the solution was added to the microspheres. The effects of time, temperature and pH are illustrated in Figs. 4, 5 and 6. Labeling carried out at higher phosphate concentrations was less efficient.

The ^{113m}In was not removed from the microspheres with repeated saline washes when the microspheres were labeled by the procedure outlined in Fig. 3. When these microspheres were injected intravenously, over 94% of the radioactivity was concentrated in the lungs (Table 1). In some of the preparations which were heated to too high a temperature, the liver activity was increased, sometimes to 20–30% of the injected dose. Microspheres labeled in more basic phosphate solutions also produced higher liver concentrations of radioactivity.

On microscopic examination, the labeled microspheres remained unclumped and had the same uniform diameter and brown color as before labeling.

DISCUSSION

The labeling procedure that we are describing is fast and simple and provides a stable microsphere of high specific activity with the same properties as

TABLE 1. DISTRIBUTION OF 0.1 mg ^{113m}In MICROSPHERES IN MICE

Time sacrificed	No. of mice	% dose lung	% dose liver	% dose carcass
10 min	3	94.6	0.48	4.86
1 hr	3	91.2	1.09	7.67
3 hr	3	88.7	1.28	9.98
5 hr	3	85.5	1.30	13.18

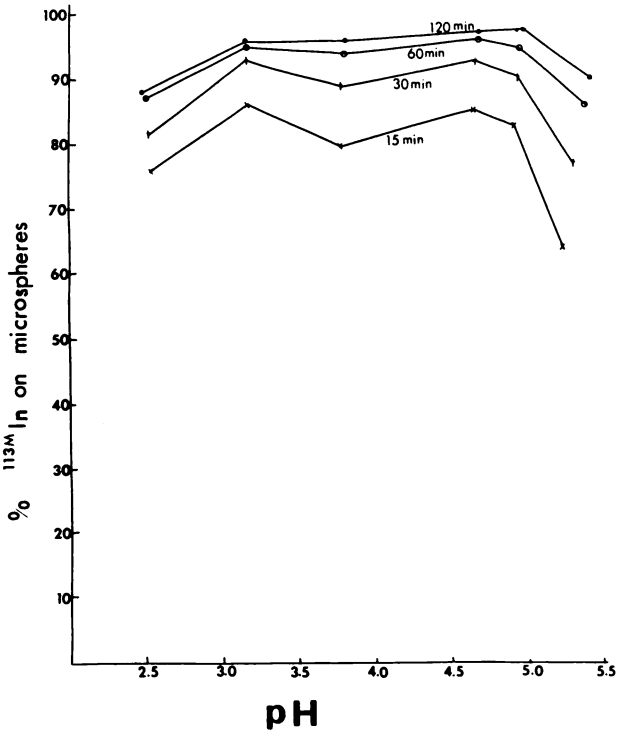

FIG. 6. Microspheres were incubated with ^{113m}In at 75°C in phosphate solutions varying in pH from 2.5 to 5.5 and labeling measured as function of time.

the original microspheres. From our previous studies, we knew that indium could be incorporated in iron hydroxide particles (*3*) which were useful as a lung scanning agent. Thus human serum albumin microspheres containing iron hydroxide were chosen as the starting material.

One of the first problems which had to be solved was to disperse the microspheres in the tagging solution, because they tended to float on the surface. The microspheres did not disperse well in an aqueous solution, but the addition of a small amount of 5% Tween 80 overcame this problem. Various concentrations of other wetting agents such as Triton, propylene glycol and carboxy-methyl-cellulose were also tested, but the labeling was not as good.

The most efficient and stable labeling was achieved when the pH was adjusted to pH 3 with 0.2 *M* Na_2HPO_4. Only 73% labeling was achieved when the solution was adjusted to pH 3 with NaOH, presumably because the addition of 10 mg of microspheres to this solution caused the pH to rise to 4.9, which was too high. The maximum labeling achieved with Na_2CO_3 solutions, regardless of pH, was 67%.

With the method outlined in Fig. 3, particles of iron hydroxide were readily labeled with ^{113m}In, but microspheres that did not contain iron hydroxide were not. Phosphate was not necessary, but the most efficient and stable labeling was always achieved when disodium phosphate was used. At pH's greater than 4.5, tests in mice indicated that this tagging was not as stable *in vivo* as when the microspheres were tagged at pH 3–4.5. Although the nature of the binding of the ^{113m}In to the microspheres was not established, it probably represents the adsorption of the insoluble ^{113m}In hydrous oxide or phosphate by the iron particles within the microspheres.

Microspheres tend to settle quickly in a vial or syringe, a characteristic which made injections of precise amounts difficult. Therefore, it was necessary to shake the vial containing the microspheres immediately before injection. The syringe was rinsed by withdrawing blood into the syringe several times before the needle was withdrawn. The activity in the syringe was measured before and after injection to obtain an accurate measure of activity injected.

For human studies, 5–10 mg of microspheres were usually labeled with 1–3 mCi of ^{113m}In. Much higher specific activities could be achieved if desired. The number of microspheres as well as the amount of radioactivity injected is important. If too few microspheres are used, the statistical variation of the distribution of the microspheres within the organ of interest may become significant. Since the number of microspheres varies as a cubic function of their radius, the appropriate weight of microspheres depends on the size of microspheres and the anticipated volume of distribution. For example, we estimate that approximately 100,000 particles are needed to avoid statistical variations in the pattern of distribution in the lungs of an adult. If microspheres with a diameter of 35 microns are used, then at least 3 mg would be necessary (Fig. 2). However, if 15-micron microspheres are used, then only 0.25 mg would be necessary.

CONCLUSIONS

A method for labeling human serum albumin microspheres containing iron hydroxide with ^{113m}In has been developed. The labeling efficiency of this procedure is 94% or better, and the product is biologically stable. The overall procedure requires about 45 min and can be carried out under sterile conditions so that radiopharmaceutical-grade preparations can be achieved.

ACKNOWLEDGMENT

This work was supported by USPHS Grant #GM 10548.

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