LABELING IRON-FREE ALBUMIN MICROSPHERES WITH ^{113m}In

Julia W. Buchanan, Buck A. Rhodes, and Henry N. Wagner, Jr.

Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland

Albumin microspheres, 15–30 microns in diameter, are a useful radiopharmaceutical for lung scanning and other studies of the circulation (*1,2*). They can readily be labeled with ^{99m}Tc using a kit procedure (*3*). Human serum albumin microspheres containing iron hydroxide have been labeled with ^{113m}In (*4*), but those without iron have only been labeled with ^{99m}Tc. This paper presents a method for labeling microspheres with ^{113m}In without the necessity of including iron in the original microspheres.

The advantages of iron-free microspheres labeled with ^{113m}In are: (A) iron-free microspheres have been extensively used clinically for over 2 years, and their in vivo behavior is well defined; (B) the same lot of microspheres can be labeled with both ^{99m}Tc and ^{113m}In; and (C) since iron is slowly cleared from the lungs, use of non-iron containing microspheres avoids any possible undesirable effect of the iron (*1,5*). This should make possible a wider use of microspheres, especially in areas of the world where ^{113m}In is a more economical nuclide, or where only iron-free microspheres are available.

MATERIALS AND METHODS

The ^{113m}In was eluted from a ^{113}Sn-^{113m}In generator with 0.05 *N* HCl. In this study several New England Nuclear generators and one from Commissariat à l'Énergie Atomique, Département des Radioéléments, France were used. The 3M Company supplied the human serum albumin microspheres, which were 15–30 microns in diameter. The Hopkins tagging vials were supplied by the Wheaton Glass Co., Millville, N.J. and contained a fritted glass filter. These double-closed vials are illustrated in Fig. 1. One molar acetate buffer was prepared at pH 5.5 by diluting 6.8 ml of 2 *M* acetic acid and 43.2 ml of 2 *M* sodium acetate to 100 ml. A 0.1% solution of Tween 80 (polysorbate 80) was prepared using distilled water. The washing solution was 0.01% Tween 80 in 0.9% saline with the pH adjusted to 5.5. A 0.05 *M* solution of borax was prepared by dissolving 19.05 gm of sodium tetraborate in 1,000 ml. The pH of this solution was adjusted to 11 with 1 *N* NaOH.

Figure 1 diagrammatically illustrates the procedure for labeling the plain microspheres with ^{113m}In. The volume of ^{113m}In, eluted from a ^{113}Sn-^{113m}In generator, was adjusted to 10 ml with 0.05 *N* HCl. This was added to a vial containing 1 ml of acetate buffer, pH 5.5, and 1 ml of 0.5 *N* NaOH. One tenth milliliter 0.1% Tween 80 was added. The contents of the vial were transferred to a tagging vial containing 10 mg of microspheres. The pH was 5.5. The vial was placed in an ultrasonicator for 30 min. Then 1.5 ml of 0.05 *M* borax, pH 11 was added, bringing the pH of the labeling solution to 9.5 and this was boiled 10 min. The supernate was removed from the vial, and the labeled microspheres were washed with saline and Tween 80. The microspheres were resuspended in saline-Tween 80 solution for injection.

The percent labeling was determined by centrifuging a 1-ml sample, removing the top 0.5 ml of supernate, counting the two aliquots, and calculating:

$$\% \text{ labeling} = \frac{\text{cpm bottom} - \text{cpm top}}{\text{cpm bottom} + \text{cpm top}} \times 100.$$

The percent labeling of the final product was also obtained by counting the vial before and after washing.

The stability of the label was determined by incubating 1 ml of the labeled microspheres with 1 ml of normal human serum for 30 min at room temperature. The percent binding was then determined and compared with that of the microspheres which had not been exposed to serum.

Received Jan. 27, 1971; revision accepted March 20, 1971.

For reprints contact: Julia W. Buchanan, The Johns Hopkins Medical Institutions, Dept. of Radiological Sciences, 615 N. Wolfe St., Baltimore, Md. 21205.

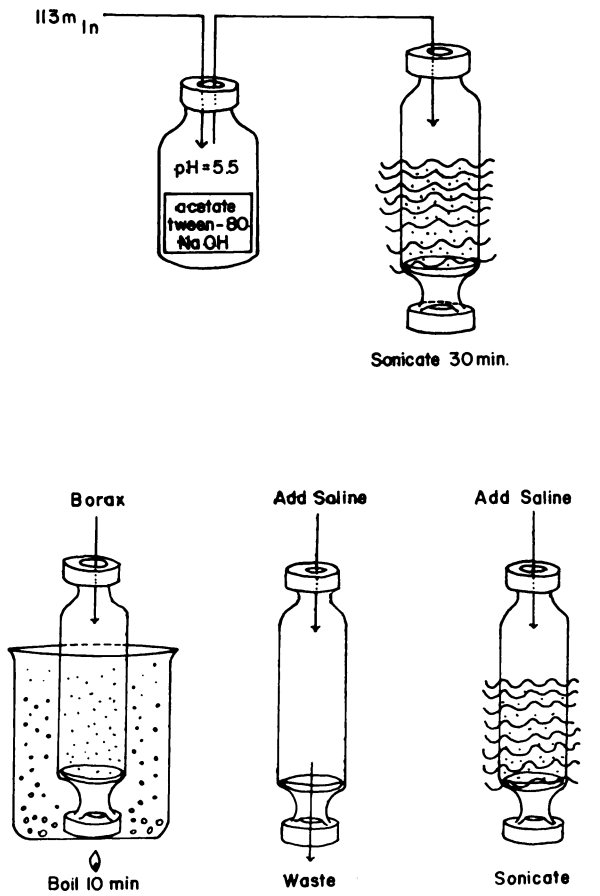

FIG. 1. Procedure for labeling iron-free microspheres with ^{113m}In.

1. Add ^{113m}In (in 10 ml 0.05 *N* HCl) to vial containing 1 ml of 1 *M* acetate buffer, pH 5.5; 0.1 ml 0.1% Tween 80 and 1 ml 0.5 *N* NaOH.
2. Transfer solution to Hopkins tagging vial containing 10 mg microspheres. Incubate 30 min in ultrasonic bath.
3. Add 1.5 ml of 0.05 *M* borax, pH 11. Heat 10 min in boiling water.
4. Remove tagging solution from below filter frit. Add 10-ml saline-Tween 80 solution.
5. Remove wash solution. Add 5–10-ml saline-Tween 80 solution. Sonicate to resuspend microspheres before injecting.

Distribution studies in mice were performed on 20–30-gm albino males. One mouse was injected through the tail vein and sacrificed immediately as a standard. The others were also injected intravenously and sacrificed 30 min later. During this 30-min period each mouse was placed in a large plastic test tube, and the total-body radioactivity was measured in a 5-in. well scintillation detector. The mice were sacrificed, and a sample of blood, the liver, lungs, kidneys, and the tail were removed. The residual activity of the carcass was also measured. The percent dose in the carcass was calculated as

$$\%\ \text{dose in carcass} = \frac{\dfrac{\text{cpm carcass}}{\text{cpm standard mouse}}}{\dfrac{\text{cpm mouse before sacrifice}}{\text{cpm standard mouse}}} \times 100.$$

The percent dose in each organ was determined and corrected as follows:

$$\%\ \text{dose organ} = \left(\frac{\text{cpm organ}}{\Sigma\text{cpm all organs}}\right) (100\% - \%\ \text{dose carcass}).$$

The percent of the dose in a known aliquot of blood was found and calculated for the total blood, assuming the blood volume to be 7% of the body weight. If more than 2–3% of the dose remained in the tail, the percent in the organs was corrected for this.

RESULTS

Factors which were found to affect the initial labeling of the microspheres with ^{113m}In were (A) the duration of the reaction, (B) the pH, (C) the concentration of the acetate buffer, and (D) the quantity of microspheres.

Figure 2 shows the increase in labeling as a function of time. Thirty minutes was selected as the optimum time, considering the 1.7-hr half-life of ^{113m}In. The reaction was carried out in an ultrasonicator where the temperature of the water was 48°C. The ultrasonication was necessary to disperse the microspheres and resulted in a higher percent labeling compared with a control reaction at room temperature with no agitation.

The percent labeling by ^{113m}In at various pHs is shown in Fig. 3. A pH of 5.5 was optimal for labeling. In the initial experiments NaOH was used to adjust the pH of the ^{113m}In. To control the pH more accurately and to make the method adaptable to a kit procedure, a buffer was used. Acetate proved to be a suitable buffer for this purpose if the final concentration was kept at 0.1 *M* or below. If the molarity of the acetate was increased, the labeling was in-

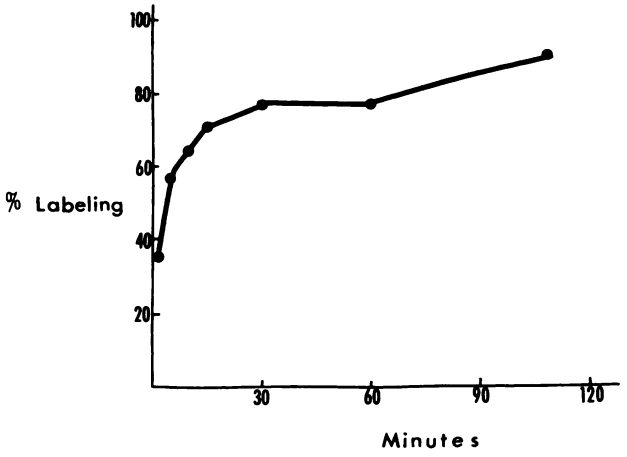

FIG. 2. Microspheres were incubated with ^{113m}In in acetate buffer, pH 5.5 in ultrasonicator. One-milliliter samples were removed at various time intervals and percent labeling determined.

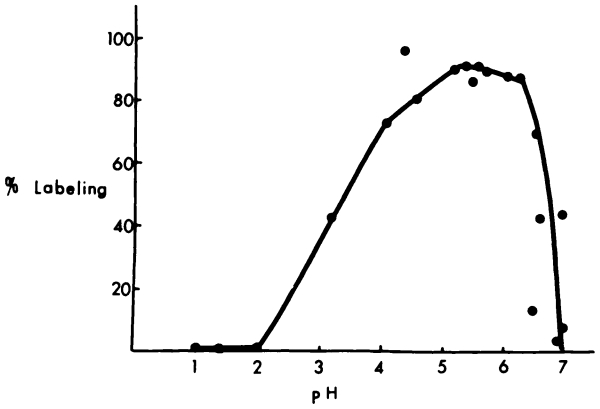

FIG. 3. Microspheres were incubated with ^{113m}In at varying pH for 30 min.

hibited. In the procedure illustrated in Fig. 1, 1 ml of 1 *M* acetate buffer is diluted to 12 ml, producing a final concentration of 0.08 *M*. This, with the additional 0.5 *N* NaOH, provided adequate control of the pH at 5.5. In this procedure 10 ml of 0.5 *N* HCl containing the ^{113m}In was buffered, making a high-specific-activity label possible.

If the microspheres were labeled at pH 5.5 and the pH was then changed by adding HCl or NaOH, some of the ^{113m}In came off the microspheres. This was more pronounced in acidic compared with basic solutions (Fig. 4). The original labeling was 80–95% at pH 5.5 and the unbound ^{113m}In could be removed by washing with saline. However, the label was not stable when the microspheres were incubated with serum. In the presence of serum, the ^{113m}In on the microspheres were reduced from 92% to 38% in 30 min. The lung concentration in mice was only 39% of the injected dose. The blood activity remained high. The lung-to-liver ratio was 7:1.

The stability of the label was increased by raising the pH of the tagging solution from 5.5 to 9.5 and boiling for 10 min. When this was done and the labeled, washed microspheres were incubated with serum, the ^{113m}In remained with the microspheres (Fig. 5). The lung concentration in mice was 70%, and the lung-to-liver ratio 11:1.

Borax was used to keep the pH at 9.5 during the stabilizing step. Another buffer, carbonate-bicarbonate, was evaluated for this purpose and found to be unsatisfactory (Table 1).

The stabilizing procedure decreased the overall yield from 81% to 61%. Since the free ^{113m}In was readily removed by washing the microspheres, this did not alter the final product. The special Hopkins tagging vials were convenient for removing the unbound radioactivity.

The ^{113m}In-labeled microspheres were injected into dogs. Five minutes after injection, 5.8% of the dose remained in the blood. This rose to 13.4% at 30 min and 27.5% at 60 min. Sixty minutes after injection the lung-to-liver ratio was 10:1. Satisfactory lung scans were obtained up to 90 min after injection.

This procedure for labeling the microspheres with ^{113m}In has been used with the kits supplied by the 3M Company for labeling with ^{99m}Tc. These kits contain thiosulfate, which was shown not to interfere with the ^{113m}In labeling. Using these kits, the initial binding at pH 5.5 was only 59% and after boiling at pH 9.5 was reduced to 31%. However, after washing, the ^{113m}In label was not removed from the microspheres by serum, and the lung concentration was equal to that of the usual method. The poor efficiency of the labeling was due in part to the fact that the 3M kits contain 5 mg of microspheres instead of 10 mg.

The overall yield of this method averaged 61%. Using the eluate of a 50-mCi generator, the specific

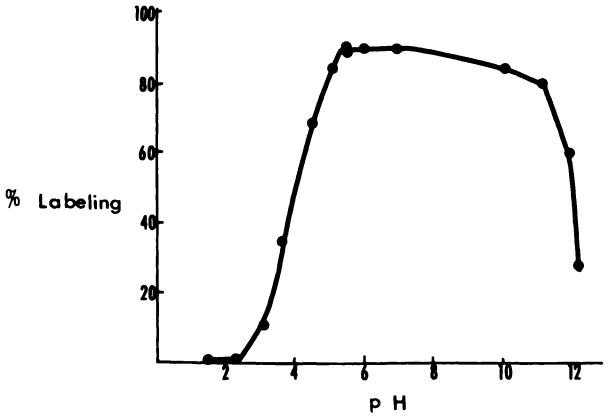

FIG. 4. Microspheres were labeled with ^{113m}In at pH 5.5; then pH was readjusted with HCl or NaOH and percent labeling determined.

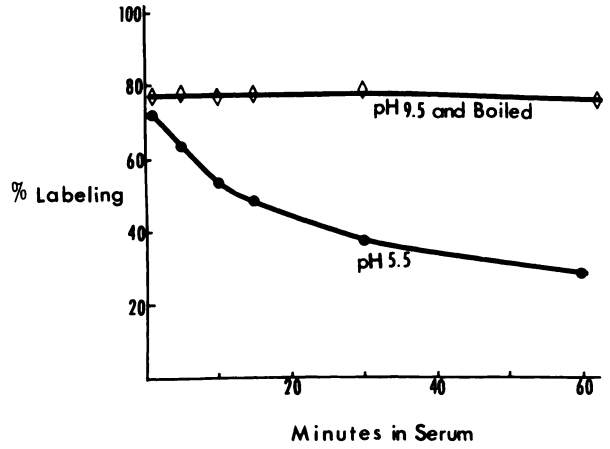

FIG. 5. Microspheres were labeled with ^{113m}In at pH 5.5. Labeled microsphere suspension was divided and pH of half of suspension raised to 9.5 and boiled for 10 min. Four milliliters of each aliquot were incubated with 4 ml of normal human serum at room temperature. Percent labeling was determined at various times.

TABLE 1. DISTRIBUTION OF ^{113m}In-MICROSPHERES IN MICE 30 MIN AFTER INTRAVENOUS ADMINISTRATION

	Procedure	No. of mice	% of injected dose				
			Lungs	Liver	Blood	Kidneys	Carcass
I.	pH 5.5	12	33.8	5.3	16.4	6.7	37.8
II.	pH 5.5 → 9.5 NaOH	18	65.0	9.2	6.7	2.6	16.5
III.	pH 5.5 → 9.5 carbonate-bicarbonate	6	34.9	20.2	12.5	4.8	27.6
IV.	pH 5.5 → 9.5 borax	12	73.4	10.7	5.6	1.7	8.6
V.	3M kit pH 5.5 → 9.5 borax	3	71.4	6.0	7.5	3.7	11.4
VI.	Control: no microspheres pH 5.5 → 9.5 borax	3	2.8	83.9	3.2	1.3	8.8

activity of the final product can exceed 1 mCi/mg of microspheres. The yield of the ^{113m}In labeling of the non-iron microspheres was not as high as with those containing iron. The lung-to-liver ratios in mice were lower than those previously reported (*4*) but were still within acceptable limits.

The yield of the labeling was somewhat variable from day to day. The reasons for this were apparently related to variations in the quality of the ^{113m}In-eluate. Tests showed that small amounts of either carrier indium or tin interfered with the labeling.

DISCUSSION AND CONCLUSION

In the pH range between 3 and 7, ^{113m}In is reversibly bound to albumin microspheres, with the highest binding occurring when the pH is 5.5. If the pH is too basic initially, the ^{113m}In may precipitate before binding to the microspheres. This is inhibited by the presence of acetate ions.

When albumin microspheres labeled with ^{113m}In at pH 5.5 are exposed to serum, the ^{113m}In is released from the microspheres. The indium in this form apparently has more affinity for the serum transferrin than for the microspheres. However, if the ^{113m}In is bound to the microspheres at pH 5.5 and then the solution made alkaline and boiled, the ^{113m}In on the microspheres is converted to a form which is less readily released to serum transferrin. The labeled microspheres stabilized in this manner gave a radiopharmaceutical suitable for lung scanning or circulation studies.

In conclusion, a method for labeling iron-free human serum albumin microspheres with ^{113m}In has been developed. The method can be carried out under sterile conditions using kits so that the labeled microspheres are suitable for human use. The yield of the procedure averaged 61% and the final product contained less than 2% unbound radioactivity. Lung scans in animals have been obtained up to 90 min after the injection of the ^{113m}In-labeled albumin microspheres. Now it is possible to label iron-free microspheres with either ^{99m}Tc or ^{113m}In. Also the same lot of microspheres can be used for dual tracer studies.

ACKNOWLEDGMENT

This work was supported by USPHS Grant GM10458.

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