jnm/ RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

TAGGING OF IRON OXIDE PARTICLES WITH ^{99m}Tc FOR USE IN THE STUDY OF DEPOSITION AND CLEARANCE OF INHALED PARTICLES

Frank J. Hass, Peter S. Lee, and Ruy V. Lourenço

University of Illinois Abraham Lincoln School of Medicine, University of Illinois Hospital, and Veterans Administration West Side Hospital, Chicago, Illinois

This paper describes a method of tagging iron oxide aerosol particles with ^{99m}Tc. The tagging is achieved by reducing the heptavalent pertechnetate form to its reduced state using stannous chloride in a nitrogen atmosphere. Tests by ultracentrifugation and paper chromatography show that the tagging is virtually complete. Separation of the tag from the particles in sputum is shown to be minimal. Monodisperse aerosols of the tagged particles are produced with a spinning-disk atomizer. (Deposition and clearance of the particles in the airways and lungs is easily followed by scintigraphy.) Factors affecting attachment and separation of the tag are discussed.

Radioactive aerosols are used in the study of deposition and clearance of particles in the lung (*1–5*). Technetium-99m particles are suitable for these studies because the nuclide has a desirable half-life of 6 hr and is a gamma-emitter; these factors favor a low radiation dose per unit of activity.

Since the pattern of particle deposition—and hence the subsequent clearance (*3*)—is known to depend upon particle size (*6,7*), it is important to use monodisperse particles in the study of their deposition and clearance. Many previously reported methods of tagging iron oxide particles with ^{99m}Tc were developed to study pulmonary perfusion (*8–11*). With these methods, the iron oxide aggregates tagged with ^{99m}Tc are too large (10–50 microns) and heterodisperse (*8,9*) for inhalation studies.

Much of the literature is concerned with the tagging efficiency of ^{99m}Tc, i.e., the amount of ^{99m}Tc which initially tags to the particles (*8,9,11,12*), and very little has been reported about the ability of ^{99m}Tc to maintain the tag with the particles. Darter and Ackerman (*13*) reported on the separation of ^{99m}Tc tag from iron hydroxide macroaggregates. They determined the amount of unbound ^{99m}Tc for up to 48 hr after preparation. However, these experiments were performed on large particles (10–15 microns) and the solution was prepared and stored under vacuum. Such conditions are expected to decrease tag separation, since the large particle size would reduce the total surface area for the same amount of material and the exclusion of oxygen is known to greatly diminish tag separation (*11,13*). Actually, very little has been published about the separation of tag after aerosol generation and inhalation.

We wish to report a tagging method for monodisperse iron oxide particles of intermediate size (2–4 microns) which results in a high tagging efficiency and minimal separation of the tag.

MATERIALS AND METHODS

Preparation. Technetium-99m was eluted from a ^{99m}Tc generator with 10 ml of deoxygenated 0.1 *N* HCl. The eluate, containing $^{99m}TcO_4^-$, was passed through a cation exchange column (AG 50W-X8, 50–100 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, Calif.) to remove any cationic impurities (*14*). A portion of the eluate was then evaporated to dryness, with the amount depending on the required radioactivity. Deionized and deoxygenated water was added to obtain 6 ml of solution. The water was deoxygenated by boiling and letting it cool while bubbling nitrogen through it. An iron oxide sol

Received April 24, 1975; revision accepted Aug. 26, 1975.

For reprints contact: F. J. Hass, Sect. of Pulmonary Diseases, Dept. of Medicine, Univ. of Illinois Hospital, 840 S. Wood St., Chicago, Ill. 60612.

suspension (A. J. Lanza Research Laboratories, Tuxedo, N.Y.) was then added and the mixture was deoxygenated by passing nitrogen through it and keeping it in a nitrogen atmosphere. The amount of the iron oxide sol added depended upon the particle size desired. We used a final concentration of 330 μg/ml. The more iron oxide present, the larger are the particles generated in the spinning-disk atomizer (*15*).

One-half milliliter of a freshly prepared solution of stannous chloride in 0.1 *N* HCl (4 mg/ml), mixed in a nitrogen atmosphere, was added to the deoxygenated iron oxide mixture. The resulting mixture was left standing in a nitrogen atmosphere for 5 min, and 13 ml of deionized and deoxygenated water was added. The stannous chloride concentration at this point was 100 μg/ml. This solution was then dialyzed with deionized and deoxygenated water using a Biofiber No. 80 beaker (Bio-Rad Laboratories), which has a molecular-weight cutoff of 30,000. Before dialysis the solution was acidic (pH $\simeq$ 1.5) to prevent hydrolysis of the stannous chloride. Dialysis was stopped when the pH reached a constant level comparable to that of deionized water, pH $\simeq$ 5. The solution was then passed through an anion exchange column (AG1-X8, 50–100 mesh, chloride form, Bio-Rad Laboratories) to remove any remaining $^{99m}TcO_4^-$. Ten milliliters of this solution was used for atomization.

Generation and characterization of particles. Aerosol particles were generated using a spinning-disk atomizer (*4,15*). Particle size was controlled by adjusting either the iron oxide sol concentration or the disk speed. The particles were collected with a cascade impactor (C. F. Casella and Co., London, England) and the particle size distribution was determined by comparing the particles with a Porton graticule under an optical microscope. Once the size distribution was determined, monodispersity (*16*) and other important parameters (geometric mean, geometric standard deviation, arithmetic mean, mass median diameter, etc.) can be readily determined (*17*).

Test for tagging efficiency. The tagging efficiency of ^{99m}Tc to the particles in the iron oxide sol suspension before atomization was determined using two different methods: ultracentrifugation and paper chromatography. In the ultracentrifugation method, a 2-ml sample of the prepared suspension was taken and the amount of radioactivity determined. It was then centrifuged for 15 min at 26,000 G and an aliquot of the supernatant was taken and counted for radioactivity, thus permitting the amount of untagged $^{99m}TcO_4^-$ to be determined. In the paper-chromatography method, Whatman No. 1 paper and a methanol solvent (85% by volume) were used. The radioactivity was measured at the origin (where the particles remained) and along sections of the paper strip to determine where the free $^{99m}TcO_4^-$ had migrated. The percent of free $^{99m}TcO_4^-$ was then calculated. The position of the $^{99m}TcO_4^-$ zone was determined by running a control strip with only free $^{99m}TcO_4^-$ present.

Test for separation of tag. During atomization in the spinning-disk generator, samples of the tagged particles were collected by suction on a filter disk with a pore size of 0.45 microns. Two pieces were cut from the filter disk and the amount of radioactivity on each was measured with a well scintillation counter. Each piece was then placed in a separate test tube and soaked in either normal saline or sputum from a patient with chronic bronchitis. The particles were soaked for 2–3 hr in their respective solutions and then centrifuged at 26,000 G. In order to facilitate the centrifugation and filtration of the sputum after soaking, the sputum was broken down to a more liquid consistency by the addition of a small amount of pure 2-mercaptoethanol (final concentration, 2% by volume). The supernatant was passed through a 0.45-micron filter disk to remove any remaining particles. The filtrates were counted to determine the radioactivity present, and the amount of $^{99m}TcO_4^-$ that had separated from the particles was calculated. Because of the large differences between the radioactivity of the particles and the separated radioactivity in the supernatant, the deadtime of the counting system used had to be taken into account; this was determined by the method of paired sources (*18*).

RESULTS

The above method resulted in a very high efficiency of initial binding of ^{99m}Tc to the iron oxide sol suspension. Both the centrifugation and paper-chromatography tests produced similar results: less than 2% of the $^{99m}TcO_4^-$ remained untagged to the iron oxide sol.

The results of the paper-chromatography tests showing the percentage of unbound $^{99m}TcO_4^-$ are shown in Fig. 1B (Fig. 1A shows the control with free pertechnetate only). Dialysis prior to atomization removed the remaining 2% of the untagged $^{99m}TcO_4^-$ and any other ions present. The centrifugation and paper-chromatography tests both showed that this process removed virtually all of the free $^{99m}TcO_4^-$. The results of paper chromatography are shown in Fig. 1C.

Once the iron oxide sol has been tagged, the main concern is the ability of the tag to remain bound to the particles, not only through the atomization process but also in the in vitro or in vivo environment.

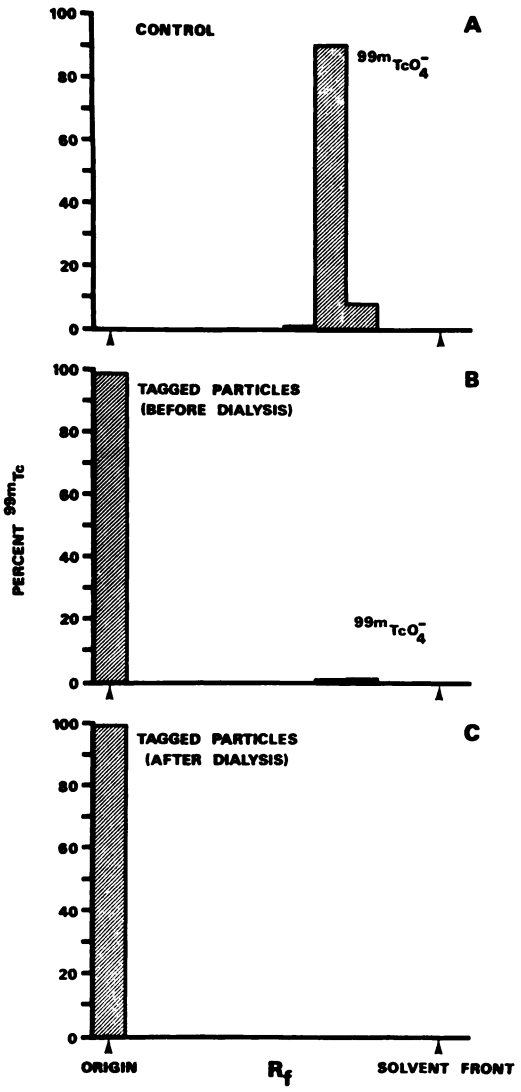

FIG. 1. Paper-chromatographic determination of unbound $^{99m}TcO_4^-$ before and after dialysis. (A) Histogram of control solution of free $^{99m}TcO_4^-$. (B) Histogram of tagged particles before dialysis shows that 98.4% of ^{99m}Tc is bound, with minimal free $^{99m}TcO_4^-$. (C) Histogram of tagged particles after dialysis shows that approximately 100% of ^{99m}Tc is bound.

After atomization, the 2-micron particles were collected and soaked in normal saline or in sputum to test for tag separation. The results showed an average separation of 1.5% when soaked in sputum and of 5.9% when soaked in normal saline.

The tagged particles were inhaled by a volunteer of normal health and clearance was followed with a scintillation camera. Three hours after inhalation, a blood sample was drawn to check for nuclide separation and absorption into the blood stream. The blood sample showed no increase in radioactivity. Further evidence of firm binding can be seen in scintigrams taken 5 hr apart (Fig. 2); these show good definition of the lung outline, with an excellent target-to-background ratio.

DISCUSSION

When the ^{99m}Tc generator is eluted, the technetium is in the heptavalent pertechnetate form. Since chemisorption of heptavalent pertechnetate on iron hydroxide is unlikely (*19*), the ability of ^{99m}Tc to tag to the iron oxide particles depends on its being in the reduced state (*8,20*). The high tagging efficiency of the present method derives from the reduction of $^{99m}TcO_4^-$, accomplished by the addition of stannous chloride. The respective half-cell potentials (*21*) are

$$Sn^{+2} = Sn^{+4} + 2e^- \quad (0.1\ N\ HCl) \qquad E^0 = -0.070$$

$$TcO_2 + 2H_2O = TcO_4^- + 4H^+ + 3e^- \qquad E^0 = -0.738$$

$$2H_2O = O_2 + 4H^+ + 4e^- \qquad E^0 = -1.229$$

Reoxidation must be considered since it might cause the separation of the tag from the particles. This was confirmed by adding a small amount of a reducing agent (0.6 mg/ml of $SnCl_2$) to the saline solution, whereupon the amount of separation of the tag was drastically reduced to less than 1.5% in all cases. Since some proteins act as chelating agents with transition metals, sputum proteins may account for the lesser tag separation in sputum as compared to saline (*22,23*).

A small percentage of separation, however, seems inevitable. It could be explained by the intrinsic nuclear properties of the ^{99m}Tc itself. Technetium-99m emits 1.35 Auger electrons with each decay event (*24*). These electrons result in a highly charged state of the molecule (*25*), creating a redistribution of charge that could cause separation through elec-

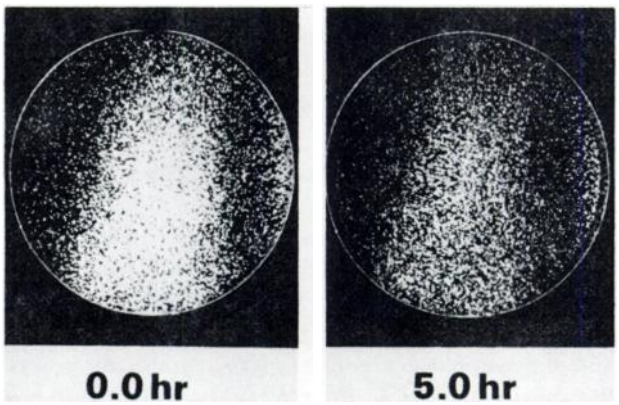

FIG. 2. Aerosol inhalation scintigrams of right lung using ^{99m}Tc-tagged iron oxide particles. Pictures taken 5 hr apart give no indication of significant tag separation, as evidenced by good lung definition and good target-to-background ratio. About 14 μCi activity was deposited originally in right lung, resulting in dose of about 4 mrad.

trostatic repulsive forces. Whatever its cause, we believe this separation to be insignificant, as evidenced by the lack of radioactivity in the blood sample and by the good target-to-background ratio in the scintigrams (Fig. 2). If there were significant amounts of free technetium, the target-to-background ratio would be much poorer (*26*).

In summary, we feel that the procedure described is useful for the tagging of ^{99m}Tc to iron oxide particles. This procedure results in highly efficient tagging with minimal tag separation. It gives us a useful tool with which to study the deposition and clearance of inhaled particles in the human lung.

ACKNOWLEDGMENT

This work was supported in part by Grant HL-13824 from the National Heart and Lung Institute.

REFERENCES

1. MORROW PE, BATES DV, FISH DR, et al: Deposition and retention models for internal dosimetry of the human respiratory tract. *Health Phys* 12: 173–207, 1966

2. ALBERT RE, LIPPMANN M, SPIEGELMAN J, et al: The deposition and clearance of radioactive particles in the human lung. *Arch Environ Health* 14: 10–15, 1967

3. LOURENÇO RV: Distribution and clearance of aerosols. *Am Rev Respir Dis* 101: 460–461, 1970

4. LOURENÇO RV, KLIMEK MF, BOROWSKI CJ: Deposition and clearance of two μ particles in the tracheobronchial tree of normal subjects—smokers and nonsmokers. *J Clin Invest* 50: 1411–1420, 1971

5. LOURENÇO RV, LODDENKEMPER R, CARTON RW: Patterns of distribution and clearance of aerosols in patients with bronchiectasis. *Am Rev Respir Dis* 106: 857–866, 1972

6. LIPPMANN M, ALBERT RE: The effect of particle size on the regional deposition of inhaled aerosols in the human respiratory tract. *Am Ind Hyg Assoc J* 30: 257–275, 1969

7. GOLDBERG IS, LOURENÇO RV: Deposition of aerosols in pulmonary disease. *Arch Intern Med* 131: 88–91, 1973

8. YANO Y, MCRAE J, HONBO DS, et al: ^{99m}Tc-ferric hydroxide macroaggregates for pulmonary scintiphotography. *J Nucl Med* 10: 683–686, 1969

9. BOYD RE, ACKERMAN SA, MORRIS JG, et al: Lung scanning using ^{99m}Tc-labeled macroaggregated ferrous hydroxide (Tc-MAFH) as the perfusion agent. *J Nucl Med* 10: 737–739, 1969

10. BOYD RE: Improvements in the preparation of ^{99m}Tc-labeled macroaggregated ferrous hydroxide (Tc-MAFH). *J Nucl Med* 11: 517, 1970

11. DAVIS MA: ^{99m}Tc-iron hydroxide macroaggregates (^{99m}Tc-FHMA): Stability studies. *Radiology* 98: 448–449, 1971

12. BRUNO F, BROOKEMAN VA, ARBORELIUM M, et al: ^{99m}Tc-iron hydroxide aggregates for lung scanning. *J Nucl Med* 11: 134–137, 1970

13. DARTER EL, ACKERMAN LR: Improved binding and stability of ^{99m}Tc-iron hydroxide macroaggregates. *J Nucl Med* 12: 632–634, 1971

14. BILLINGHURST MW: Quality control of radiopharmaceutical kits. *J Nucl Med* 14: 362–363, 1973

15. MERCER TT: Production and characterization of aerosols. *Arch Intern Med* 131: 39–50, 1973

16. FUCHS WH, SUTUGIN AG: Generation and use of monodisperse aerosols. In *Aerosol Science*, Davis CN, ed, New York, Academic, 1966, pp 1–30

17. SILVERMAN L, BILLINGS CE, FIRST MW: *Particle Size Analysis in Industrial Hygiene*, New York, Academic, 1971, pp 235–259

18. FRIEDLANDER G, KENNEDY JW, MILLER JM: Statistical considerations in radioactivity measurements. In *Nuclear and Radiochemistry*, 2nd ed, New York, Wiley, 1964, pp 166–190

19. ANDERS E: *The Radiochemistry of Technetium*, Washington, D.C., National Academy of Sciences (NAS-N5) Subcommittee on Radiochemistry, National Research Council, 1960, pp 4–5

20. ECKELMAN W, MEINKEN G, RICHARDS P: Chemical state of ^{99m}Tc in biomedical products. *J Nucl Med* 12: 596–600, 1971

21. WEAST RC: *CRC Handbook of Chemistry and Physics*, 47th ed, Cleveland, Chemical Rubber, 1966, p D84

22. TETTAMANTI G, PIGMAN W: Purification and characterization of bovine and ovine submaxillary mucins. *Arch Biochem Biophys* 124: 41–50, 1968

23. LIN MS, WINCHELL S, SHIPLEY B: Use of Fe(II) or Sn(II) alone for technetium labeling of albumin. *J Nucl Med* 12: 204–211, 1971

24. DILLMAN LT: Nuclear parameters of radionuclides. MIRD Pamphlet No 4, *J Nucl Med* 10: Suppl No 2, 22, 1969

25. WEXLER S, ANDERSON GR: Dissociation of methyl bromide by nuclear isometric transition of 4.4-hr Br^{80m}. *J Chem Phys* 33: 850–857, 1960

26. SERAFINI AN, WATSON DD, NELSON JP, et al: Bone scintigraphy—Comparison of ^{99m}Tc-polyphosphate and ^{99m}Tc-diphosphonate. *J Nucl Med* 15: 1101–1104, 1974