RAPID CLOSED-SYSTEM PRODUCTION OF ^{99m}Tc-ALBUMIN USING ELECTROLYSIS

H. J. Dworkin and R. F. Gutkowski

William Beaumont Hospital, Royal Oak, Michigan

Human serum albumin (HSA) tagged with ^{99m}Tc has been shown to be a useful agent for the static and dynamic imaging of vascular and intrathecal spaces (*1–4*). It is superior to ^{99m}Tc-pertechnetate because it remains within the vascular spaces for a longer period of time, thus reducing undesirable extravascular "background" radiation; and in the instance of placental imaging it reduces the absorbed radiation dose to the fetal thyroid (*4,5*). The desirability of ^{99m}Tc-HSA is attested to by the many published methods for its preparation and use; yet it is employed clinically on an extremely limited basis. Many of the preparation methods published so far are "open" (*1,3,6–11*), that is, at some stage in the procedure, the ingredients are open to room air. Only those institutions with appropriate facilities and highly trained personnel are therefore able to produce ^{99m}Tc-HSA which is safe (sterile, pyrogen-free) for human use. All reported methods (*1–3, 6–11*) require a number of complicated manipulations either before or during the preparation of the ^{99m}Tc-HSA.

Reported below is a closed-system, sterile kit which will permit most nuclear medicine facilities to rapidly produce ^{99m}Tc-HSA which is sterile, pyrogen-free, and of high quality. The method is an adaptation of the electrolytic complexation approach reported by Benjamin et al (*10,11*).

METHODS

A closed, sterile electrolysis cell is prepared by placing two wire electrodes through the diaphragm of a 20-ml serum vial (Fig. 1). A variety of metals can be used as the electrodes. At present we favor zirconium as the anode because of its shelf life; however, iron and nickel have also been used to promote binding of ^{99m}Tc to albumin (*10*). The zirconium wire anode is about 1 in. long and 1/40 in. in diameter. A commercial grade of zirconium wire is used with the following composition: Zr 97.6%, Hf 2%, and 0.4% other impurities (C, Cr, Fe, H, and N). The wire is cut at an angle to form a point which facilitates its insertion through the diaphragm. Before use, the zirconium wire is briefly washed in a hydrofluoric-nitric acid solution (*12*). The cathode may be made from any inert conductive material such as platinum or stainless steel. At present we use zirconium wire since no distinction need then be made between anode and cathode. After the electrolytic cell is assembled, 0.85 ml of 1 *N* HCl is added and the unit is sterilized in an autoclave. These units may be sampled for sterility and pyro-

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For reprints contact: H. J. Dworkin, Dept. of Nuclear Medicine, William Beaumont Hospital, Royal Oak, Mich. 48072.

FIG. 1. Sterile electrolysis cell. Two zirconium wire electrodes are seen above rubber diaphragm. These extend through diaphragm into vial. As shown, cell contains 0.85 ml of 1 *N* HCl.

genicity and then stored for later use. There appears to be no deterioration of the sterilized units on storage.

The remaining sterile, pyrogen-free items used are: (1) ^{99m}Tc-pertechnetate in 5 ml of 0.9% saline containing the desired amount of radioactivity (without oxidant); (2) 0.10 ml of 25% human serum albumin; and (3) 1.2 ml of a bicarbonate buffer solution containing 4.2% sodium bicarbonate and 0.5 *N* sodium hydroxide (2%). When ^{99m}Tc-HSA is to be made, the 5 ml of ^{99m}Tc-pertechnetate are added to the shielded, electrolytic cell containing 1 *N* HCl. Immediately thereafter, 0.10 ml of the 25% HSA are added to the cell which is then briefly agitated. The pH of the contents of the cell at this point is approximately 1.3. The shielded vial is then inverted and the wire electrodes attached to a constant-current (approximately 100 mA) d-c power supply*. The current is then turned on for 42 sec, thus delivering approximately 4.2 coulombs of electricity to the cell (Fig. 2). The observed operating voltage is 3–4 volts. The shielded cell is continuously agitated throughout the 42 sec of current flow. Agitation is performed using a circular motion, thus keeping the electrodes immersed. The cell is then reinverted to the upright position and is permitted to stand for 30 min at room temperature. After the elapsed time the bicarbonate buffer, 1.2 ml, is added to bring the final pH of the cell contents to approximately 7.4. The final volume is approximately 7 ml. The ^{99m}Tc-HSA is now ready for human use. It has been our practice to pass this final product through a 0.22-micron bacterial filter before patient administration, although this step is probably unnecessary. Ten tests for bacterial contamination (performed before filtration) and ten additional tests for pyrogens have been negative.

The binding efficiency (BE) of the ^{99m}Tc to the human serum albumin was determined by ascending instant thin-layer chromatography (ITLC) on Gelman type S.G. media using an 85% methanol solvent. The BE is equal to the ^{99m}Tc radioactivity in the HSA peak (origin) $\times$ 100 divided by the total ^{99m}Tc radioactivity on the media strip. This method was occasionally compared with the BE obtained by protein precipitation using 5% trichloracetic acid. It is of interest that binding efficiencies similar to those reported under "Results" may be obtained by performing the electrolysis immediately before adding the ^{99m}Tc-pertechnetate and HSA.

By using specific-activity equality, one can estimate the total amount of zirconium which passes from the anode into solution. A group of zirconium wire anodes was irradiated with thermal neutrons (courtesy of John Jones, Ford Reactor, University of Michigan), producing various radionuclides of zirconium and hafnium. The specific activity of each electrode was determined by clipping off a small piece of wire before its use as the anode in the cell described above. The clipped sample which served as a standard was weighed, dissolved completely in acid, and later counted. After a typical electrolysis run (0.1 amp and 42 sec), similar volumes of the standard and the cell contents were counted (after ^{99m}Tc decay). Each was corrected for total volume, thus yielding the total radioactivity in the standard (S) and in the 7-ml volume of the cell contents (Y). To determine the milligrams of zirconium dissolved, the specific activity of the anode (cpm/mg of anode) is set equal to the specific activity of the liquid contents of the cell, yielding the following relationship:

$$\text{mg of anode in solution} = \frac{\frac{\text{mg of anode}}{\text{standard}} \times \text{Y}}{\text{S}}.$$

Since the anode is 98% zirconium, the result of the above calculation closely estimates the weight of zirconium which passed into solution as a result of electrolysis. Five independent estimations of the zirconium content in the final product were performed.

Fifteen 100-gm Yale white rats were used to examine the whole-blood disappearance of ^{99m}Tc-HSA. A dose bottle containing known amounts of ^{131}I-IHSA and ^{99m}Tc-HSA was prepared. The rats were injected i.v. with the mixture, and samples were

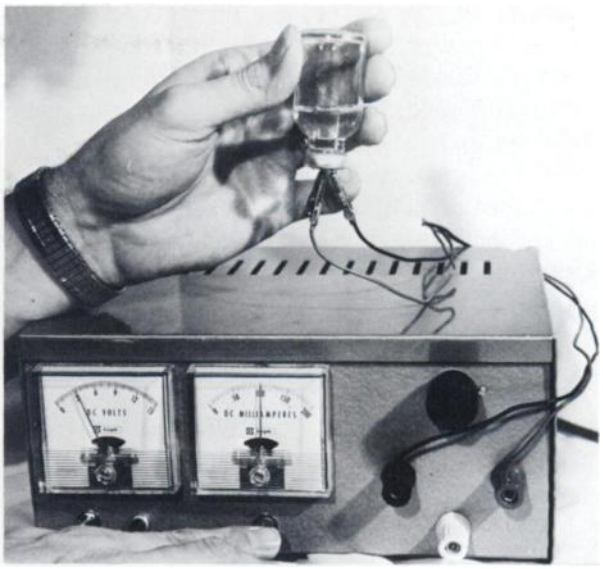

FIG. 2. During electrolysis cell is held in inverted position. The d-c power supply meters read 3.3 volts and 100 mA during 42 sec of current flow. Note that cylindrical lead shield usually used has been removed from electrolysis cell for illustrative purposes.

* Power supply designed and constructed by Ken Cook, Dept. of Applied Physics, William Beaumont Hospital.

drawn at 10, 20, 30, 60, and 90 min. After simultaneous counting, the $^{99m}Tc/^{131}I$ ratio in the whole blood was divided by the $^{99m}Tc/^{131}I$ ratio in the dose bottle. This yields a fractional (or percent) value of ^{99m}Tc per unit radioactivity of ^{131}I in the blood. This method assumes the ^{131}I present to be 100% at any given time interval. This provides a simple, geometry-independent method of comparing ^{99m}Tc-HSA blood levels with the blood levels of the better established agent, ^{131}I-HSA. Corrections were made for ^{131}I scatter in the ^{99m}Tc channel and for ^{99m}Tc decay. Using the above procedure, ^{99m}Tc-HSA prepared by two "chemical" methods (*1,13*) was also compared to the blood disappearance of ^{131}I-IHSA in 15 additional rats.

RESULTS

Over 70 batches of ^{99m}Tc-HSA have been prepared. The BE ranged from 83 to 98% with an average of 93%. The most recent modification of this kit, which is reported above, has been used in the preparation of the last 20 batches with a BE ranging from 90 to 98%, average 96%. The specific activity varies with the radioactivity added to the cell. The range is 0.6–3 mCi/mg (using a nonfractionated 300 mCi ^{99}Mo-^{99m}Tc generator). With fractionation of the eluate and using minimum quantities of albumin, an optimal specific activity of 25 mCi/mg may be attained.

Blood-flow studies and placental images using this preparation in 20 patients have been of high quality. No untoward reactions have been observed.

The zirconium content found in the final product (approximately 7 ml) is 0.91, 0.93, 0.93, 0.95, and 0.96 mg.

The whole-blood disappearance rate in rats of the intravenously administered ^{99m}Tc-HSA as a percent of the simultaneously given ^{131}I-IHSA were: 10 min—99%, 20 min—99%, 30 min—96%, 60 min—94%, and 90 min—92%. The "chemically" prepared agents disappeared at the same rate or more rapidly than the electrolytically prepared material presented above.

DISCUSSION

The closed-kit, electrolytic method reported is rapid, simple, and can routinely yield a product with more than 90% of the ^{99m}Tc present bound to the HSA. No further purification of the final product through a resin column is required. This kit is no more complex to use than other radiopharmaceutical kits currently available from commercial suppliers. Addition of the reagents to the electrolytic cell and electrolysis requires about 1 min. The 30-min waiting period after electrolysis is suggested because BE increases with time after cessation of the electric current and reaches a plateau by 30 min. If oxidants (HCLO) are present in the pertechnetate solution added to the electrolytic cell, the BE falls to about 6%.

Initially, a few dark flecks of material were noted on the bacterial filter after filtering each batch. These were presumably small pieces of material from the anode. Washing of the zirconium wire anode in the hydrofluoric-nitric acid solution before use appears to have remedied the problem.

During electrolysis a vigorous effervescent reaction is noted at the cathode. The reactions at either electrode remain somewhat obscure; however, it appears that a gas, presumably hydrogen, is given off at the cathode, about 0.4 ml. This amount of H_2 is consistent with the passage of 4.2 coulombs (42 sec $\times$ 0.1 amp). The zirconium content measured is approximately equal to that calculated with Faraday's law (about 1 mg) for the 4.2 coulombs passed (*11,14*). A thin, grey-black deposit was observed to form on the anode during electrolysis. This could represent a mixture of the hydride and oxide of zirconium back depositing on the anode (*15*), thus accounting for the small difference between our measured zirconium levels in solution of about 0.94 mg and the 1 mg estimated by calculation.

The exact mechanism whereby the zirconium ion or other metalo-ions promote the binding of ^{99m}Tc to albumin is not known; however, Benjamin et al have suggested a possible sequence of reactions not inconsistent with our observations (*11*). The electrolytically produced zirconium species which promotes such binding is transient and is not merely the zirconium ion as obtained from a variety of zirconium salts. If $^{99m}TcO_4^-$ and HSA are added to an electrolized solution (HCl and NaCl) 2 hr postelectrolysis, the BE will be reduced to below 50% compared with over 90% if the $^{99m}TcO_4^-$ and HSA are added immediately after electrolysis. If $^{99m}TcO_4^-$ and HSA are added 24 hr postelectrolysis, the BE falls to less than 1%.

The animal toxicity literature available suggests that if the entire 7 ml (1 mg of zirconium) were given to a 70-kg man, the acute toxicity "safety factor" would be in excess of 10,000 (*16–19*). The ^{99m}Tc-HSA is felt to be without significant toxicity.

SUMMARY

A closed method for the production of ^{99m}Tc-labeled human serum albumin using electrolysis is presented. This method permits the rapid production of ^{99m}Tc-labeled albumin simply and under sterile and pyrogen-free conditions. The product is of high quality, demonstrating more than 90% of

the ^{99m}Tc bound to human serum albumin in the final product. The method is easily adapted to a "kit" approach.

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