A METHOD OF PREPARING STERILE RADIOPHARMACEUTICALS USING INDICATORS

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In the preparation of some radiopharmaceuticals, it is necessary to use pH meters to control the pH of the solution before it is administered. Some of these preparations are easily sterilized by Millipore filtration. Others have a particle size too large for filtration and require autoclaving which is costly and time-consuming. Autoclaving requires from 45 min to 1 hr and since, for a given radioactive dose, the volume used will double for every half-life, time is critical when the nuclide used has a relatively short half-life as does ^{113m}In.

On the other hand, isotopic decays affect the testing: because radiopharmaceuticals labeled with short-lived nuclides can be tested only in a retrospective sense, extra care should be taken in preparing these compounds so as not to break sterility and apyrogenicity. Some radiopharmaceuticals such as albumin compounds are unstable to heat and cannot be sterilized by autoclaving. When the product cannot be autoclaved or filtered using bacterial filters (0.20–0.45-micron size), they have to be prepared aseptically.

Keeping these facts in mind, we tried a new approach to solve the problem of preparing sterile short-lived compounds when pH has to be carefully controlled. One of the problems we were faced with was the preparation of ^{113m}In-labeled macroaggregates for lung scannings.

Since it was introduced in 1964 (*1*) the agent of choice for lung scanning has been macroaggregates of ^{131}I-human serum albumin. These macroaggregates were the only reliable agent for lung scanning until the introduction of ^{113m}In-tagged ferric hydroxide particles (*2,3*). The ^{113m}In-labeled particles have the advantage of greatly increasing the photon output while reducing the absorbed radiation dose to the patient. The greatest disadvantage of the procedure described by Stern *et al* (*2*) is the final autoclaving step necessary to make sterile particles suitable for injection. The half-life of ^{113m}In is only 103 min and rapid preparation is essential to avoid unnecessary decay.

In making $Fe(OH)_3$ particles labeled with ^{113m}In, it is necessary, after adding ferric iron to the eluent from the generator, to raise the pH to 10.5–11.5 with NaOH while stirring continuously until a red-dish-brown "lake" is formed. After adding gelatin to protect the lake, the pH of the mixture must be adjusted to pH 7.5 with dilute HCl. These pH adjustments require the use of a pH meter, with the consequent breaking of sterility when the electrodes are introduced into the solution.

We felt that by using our sterile ^{113}Sn-^{113m}In generator (*4*) and a suitable dye to indicate the desired changes in pH, we could prepare sterile ^{113m}In-tagged ferric hydroxide aggregates avoiding final autoclaving.

MATERIAL AND METHODS

Several indicators with color changes over the appropriate pH ranges were investigated for practicability, usefulness and toxicity. Most of the dyes tested were of the sulfonphthalein group. Sulfonphthaleins are polyacids in which the dissociation constant of the strong sulfonic acid group is so much greater than the weak phenolic group that there is no serious interference with the latter group with which the principal color change is associated. These compounds also have more than one tautomeric form, giving rise to the possibility of more than one color change (*5*).

Of all the dyes in the sulfonphthalein group tested, we found that the 2-2′ diisopropyl-5-5′ dimethyl phenol sulfonphthalein (thymol blue) (Fig. 1) was the indicator of choice because of its physical and toxicological characteristics. The strong sulfonic acid group in thymol blue is already ionized when the phenolic group undergoes its transformations so there is a big spread between the dissociation curves in the acid and alkaline ranges as is shown in Fig. 2.

Thymol blue was tested for toxicity and its possible effects on the particles produced. Mice and rabbits were used for toxicity studies. A minimum lethal intravenous dose was not found since doses of up to 2,800 times the calculated dose for humans had no visible effects on both types of animals. Autopsy findings in the few mice that died immediately

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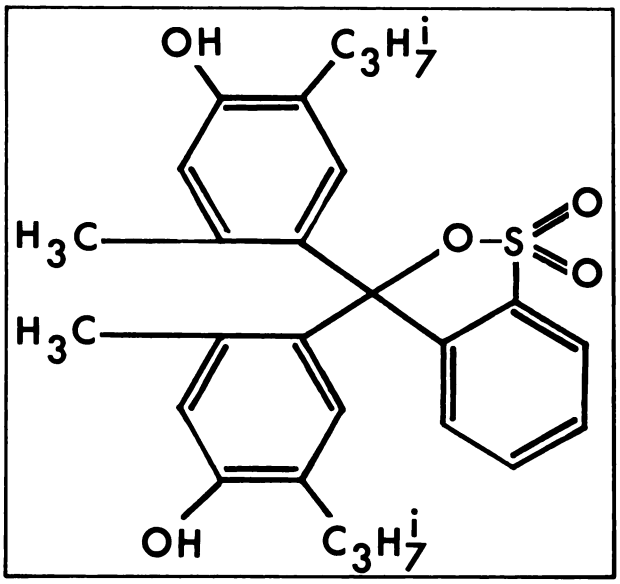

FIG. 1. Formula of thymol blue showing two phenolic and sulfonic groups. All three are readily neutralized by alkalies.

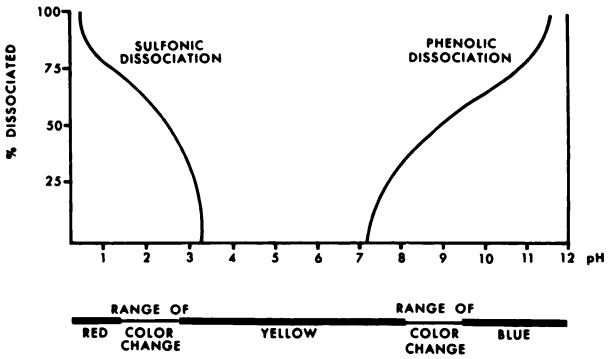

FIG. 2. Dissociation curves and color changes when pH of solution of thymol blue increases from pH 1 to 12.

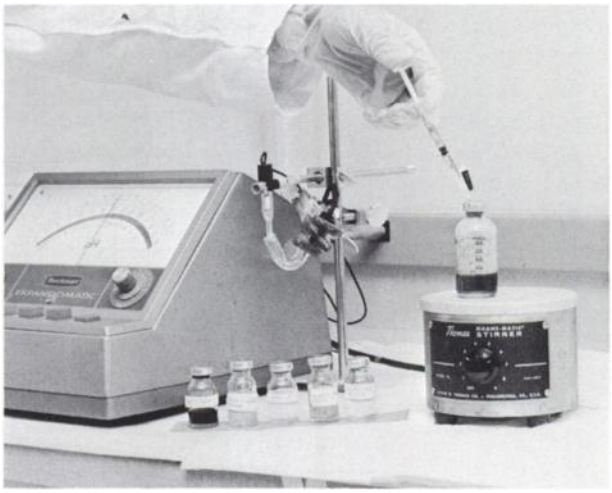

FIG. 3. Necessary reagents and equipment for preparation of sterile ^{113m}In-labeled $Fe(OH)_3$ particles.

after intravenous injection showed that pulmonary edema due to too large volume being injected was the cause of immediate death.

Histological examination of the tissues of the spleen, liver, heart, kidneys and lungs showed these organs to be normal when the animals were sacrificed 1 day and 1 week after intravenous administration of the dye solution. Six mice, injected intravenously with 2,800 times the calculated human dose, were still alive 1 month later. Histological examination of the tissues of the organs of these mice did not show any abnormality. Since a toxicity safety factor of 10^3 is usually considered safe (*2*), these toxicity tests were accepted as satisfactory.

The fate of thymol blue in the body was studied with rabbits sacrificed 1 hr after intravenous injection of large doses (30 mg) of the dye. Very little dye was found in the blood 1 hr after the dye was administered, and it was found that about 5% of the dose injected was eliminated by the kidneys and found in the urine. It has been shown by Ambrose (*6*) and Harrow (*7*) that D-glucuronic acid, together with glycine and to a lesser extent cysteine, glutamine and ornithine are used by mammals as a means of detoxicating poisonous substances that have gained access to the body. Phenol and phenol derivatives were found by these authors to be detoxicated, at least in part, by the formation of glucuronides with the D-glucuronic acid.

Other sulfonphthalein dyes are being widely used in clinical medicine. Bromosulfonphthalein (BSP) is given intravenously in doses of up to 5 mg/kg of body weight to study human liver function (*8*). Harborne (*9*) indicates that there is no evidence that BSP forms a glucuronide or a sulfonate *in vivo*. In the rat and dog BSP labeled with ^{35}S is largely excreted in the bile conjugated with glutathione. Grodsky (*10*) and Combes (*11*) reported that BSP is detoxicated and conjugated with cysteine and glutathione in the liver.

These data suggest that thymol blue is conjugated in the liver and excreted as a glutathione derivative in the bile or as a glucuronide in the urine.

PARTICLE PREPARATION

The ^{113m}In-labeled $Fe(OH)_3$ particles are prepared using sterile and pyrogen-free eluent and reagents. The eluents used in these experiments are obtained from our own sterile and pyrogen-free generator system (*4*). The reagents are prepared in advance using pyrogen-free water and chemically pure drugs; the solutions are autoclaved in small (10 or 20 ml) vials for 20 min at 150 psi and a temperature of 250°F. It is recommended to Millipore filter the thymol blue solutions to avoid decom-

position of the dye during autoclaving. It will also be necessary to filter the NaOH solution unless Pyrex glass vials are available.

Eight milliliters of eluent are collected from the sterile generator in an evacuated pyrogen-free and pre-autoclaved 30-ml vial containing a small Teflon-coated magnetic stirring bar and 0.2 ml of a solution of thymol blue (1 mg/ml). Using sterile techniques and stirring continuously with a magnetic stirrer, the following reagents are added (Fig. 3):

1. 0.2 ml $FeCl_3$ solution (1 mg/ml).
2. NaOH (2*M*) is added dropwise until a color change from yellow to brilliant blue is observed, indicating a pH of >9.6. Stir slowly 1–2 min while heating in a water bath at about 90°C.
3. 1 ml gelatin solution (20%).
4. HCl (0.05 *M*), dropwise until color changes from blue to greenish-yellow, indicating a pH of 7.5–8.0. This final pH should be confirmed using a drop or two of the solution in the microelectrodes of a pH meter.

The final product is ready for injection 10–15 min after elution of the generator.

A simplification of this method has become apparent during our observations, namely the substitution of a buffer for HCl to readjust the final pH. However, in order to keep to a minimum the addition of an alkali to form the particles of $Fe(OH)_3$, the use of an indicator is necessary.

PARTICLE CHARACTERISTICS

The possible effect of thymol blue on particle formation, the labeling with ^{113m}In and organ distribution of the particles in mice were tested.

Particles prepared using thymol blue as an indicator for pH changes did not differ from those that were prepared using the Stern *et al* (*2*) technique. The majority of the particles ranged from 20 to 40 microns in dia which is an ideal size for lung scanning. The size and uniformity of the particles were measured directly by light microscopy on a hemocytometer slide after an acid solution of ferrocyanide had been added to produce Prussian blue.

Great care must be taken when the solution is back-titrated with HCl since an excess of acidity will begin dissolving the $Fe(OH)_3$ particles and the labeled ^{113m}In will go into solution as $InCl_3$ as shown in Fig. 4. At a pH of 6.5 or greater, more than 95% of the activity is labeled to the $Fe(OH)_3$ particles. Using 0.22-micron Millipore filters, and varying the pH from 12 to 1.5 by adding 0.05 *M* HCl, it was found that the amount of unlabeled ^{113m}In increased rapidly as the pH was lowered below 6.5.

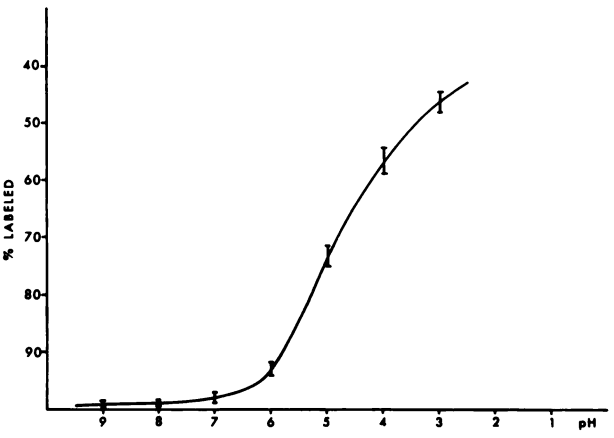

FIG. 4. Effect of pH on labeling of particles. Each point is average of five determinations. Over 95% is labeled when pH is more alkaline than 6.5.

ORGAN DISTRIBUTION IN RABBITS

Organs	% Total Dose Per Organ	% Total Dose Per Gram of Tissue
Lung	85.20 ± 3.25	5.51
Liver	1.88 ± 0.63	0.015
Spleen	0.15 ± 0.04	0.06
Kidney	0.18 ± 0.03	0.024
Blood		0.03
Urine		0.01
% of Dose Recover	87.41	
No. Replicates	6	6
No. Batches	3	3

FIG. 5. Organ distribution of ^{113m}In-$Fe(OH)_3$ in rabbits.

The organ distribution of the particles in rabbits showed that they compare favorably with the particles produced using the Stern procedure (*2*). A total of six rabbits were injected with the particles and the organ distribution studied. The results are shown in Fig. 5. The particles prepared in this way provide excellent lung scans in humans (Fig. 6).

The purpose of this paper is to present a simplified sterile method of preparation of $Fe(OH)_3$ particles labeled with ^{113m}In. Using the same principle, we believe it will be possible to prepare a large number of other radiopharmaceuticals with proven sterility and apyrogenicity. Among these, we could mention a few preparations in which ^{113m}In and ^{99m}Tc are the nuclides used. Techniques suggested by Stern *et al* (*12*) to prepare ^{113m}In-labeled compounds for blood-pool studies and brain scanning, ^{99m}Tc-labeled human serum albumin for cardiac, placental and mediastinal studies (*13,14*) and many others involve the checking of the solution's pH

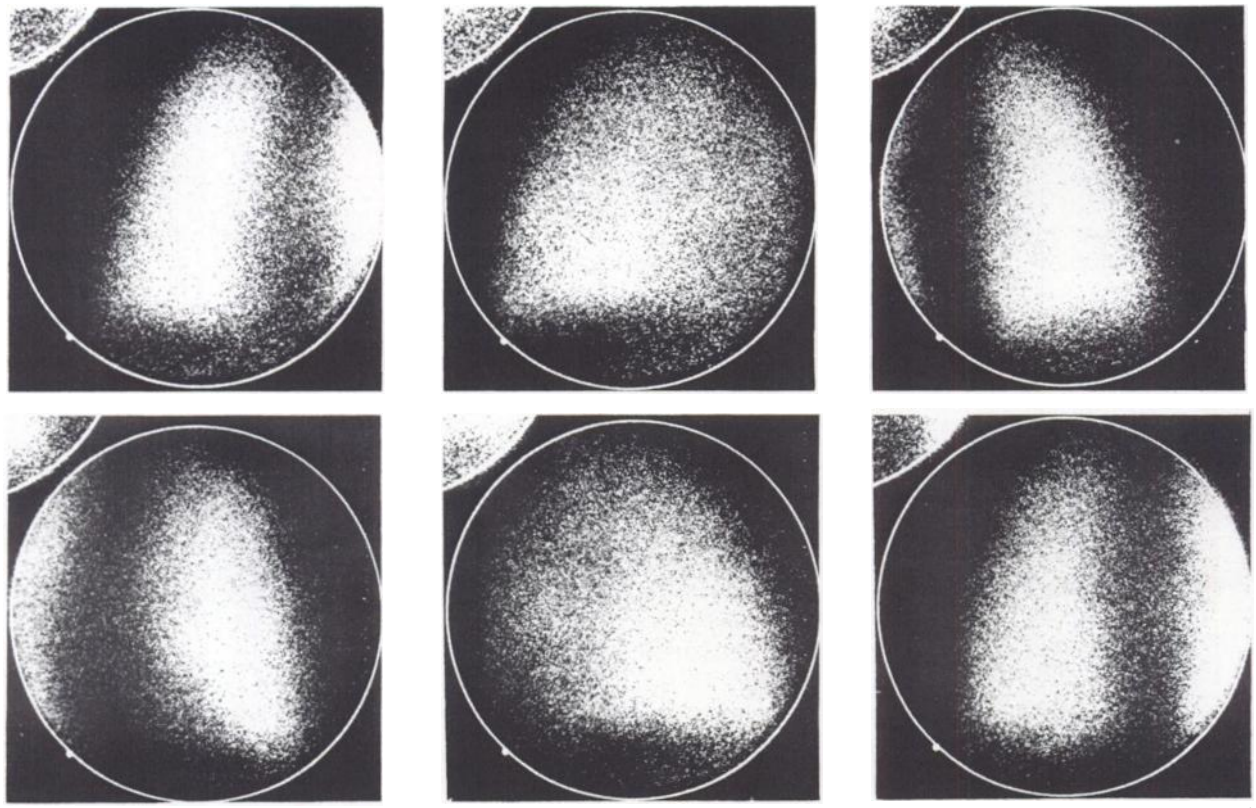

FIG. 6. Normal human lung scan with 2 mCi of ^{113m}In. Right lung in top row: AP, right lateral and PA. Left lung in bottom row: AP, left lateral and PA views.

using external determinations mostly with the help of electrodes of a pH meter, breaking sterility and apyrogenicity. It will be very simple to select a proper nontoxic internal indicator to control the required pH.

CONCLUSIONS

A simple technique based on the use of indicators to control the required pH of the solutions without breaking sterility and apyrogenicity has been described for the preparation of ^{113m}In-labeled $Fe(OH)_3$ particles. The particles prepared in the way described did not differ from particles prepared using other techniques. Size of the particles and organ distribution in rabbits has been studied and found suitable for clinical use. The greatest advantage of this procedure is to avoid autoclaving with the saving of time, which is critical when the nuclide used has a short half-life.

We believe that by using these techniques it will be possible to prepare other radiopharmaceuticals with proven sterility and apyrogenicity.

REFERENCES

1. TAPLIN, G. V., JOHNSON, D. E., DORE, E. K. AND KAPLAN, H. S.: Lung photoscans with macroaggregates of human serum radio-albumin. *Health Phys.* **10**:1,219, 1964.

2. STERN, H. S., GOODWIN, D. A., WAGNER, H. N., JR. AND KRAMER, H. H.: ^{113m}In—a short-lived isotope for lung scanning. *Nucleonics* **24**:No. 10, 57, 1966.

3. GOODWIN, D. A., STERN, H. S. AND WAGNER, H. N., JR.: Ferric hydroxide particles labeled with ^{113m}In for lung scanning. *J. Am. Med. Assoc.* **206**:339, 1968.

4. COLOMBETTI, L. G., GOODWIN, D. A. AND HINKLEY, R. L.: Preparation and testing of a sterile ^{113}Sn-^{113m}In generator. Accepted for publication in *Am. J. Roentgenol. Radium Therapy Nucl. Med.*

5. CLARK, W.: *The Determination of Hydrogen Ions.* Williams and Wilkins, 3rd Ed., 1928.

6. AMBROSE, A. M. AND SHERWIN, D. P.: Detoxication mechanisms. *Ann. Rev. Biochem.* **2**:377, 1933.

7. HARROW, B. AND SHERWIN, D. P.: Detoxication mechanisms. *Ann. Rev. Biochem.* **4**:263, 1935.

8. CANTAROW, A. AND TRUMPER, M.: *Clinical Biochemistry.* W. B. Saunders, 5th Ed., Philadelphia, 1955, p. 542.

9. HARBORNE, J. B.: *Biochemistry of Phenolic Compounds.* Academic Press, 1964, p. 243.

10. GRODSKY, G. M., CARBONE, J. V. AND FANSKA, R.: Identification of metabolites of sulfobromophthalein. *J. Clin. Invest.* **38**:1,981, 1959.

11. COMBES, B. AND STAKELUM, S.: Bilary excretion of sulfobromophthalein sodium (B.S.P.) as a mercaptide. *Clin. Res.* **8**:48, 1960.

12. STERN, H. S., GOODWIN, D. A., SCHEFFEL, U. AND WAGNER, H. N., JR.: ^{113m}In for blood pool and brain scanning. *Nucleonics* **25**:No. 2, 62, 1967.

13. WAGNER, H. N., JR.: *Principles of Nuclear Medicine.* W. B. Saunders, Philadelphia, 1968, p. 853.

14. MCAFEE, J. G., STERN, H. S., FUEGER, G. F., BAGGISH, M. D., HOLZMAN, G. B. AND ZOLLE, I.: ^{99m}Tc labeled albumin for scintillation scanning of the placenta. *J. Nucl. Med.* **5**:936, 1964.