IMPROVED BINDING AND STABILITY OF ^{99m}Tc-IRON HYDROXIDE MACROAGGREGATES

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We have used macroaggregates of iron hydroxide (^{99m}Tc-FHMA) to study pulmonary perfusion over the last 8 months. The quality of the lung scintiphotos has been generally acceptable, but some scintiphotos showed excessive free-pertechnetate judged clinically by thyroid and gastric mucosal uptake and cardiac blood-pool activity. Following normal "in air" preparation of ^{99m}Tc-FHMA, free-pertechnetate varied from 9 to 20% and increased with time following preparation. Davis has recently reported a method to largely overcome the problem of free-pertechnetate by excluding oxygen during the formation of macroaggregates (*1*). This report will describe the results of a study to determine the effectiveness of oxygen exclusion during the formation of macroaggregates using a simple vacuum technique. The stability of ^{99m}Tc-FHMA when stored at 4°C and 25°C was also determined.

MATERIALS AND METHODS

The preparation method of the macroaggregates has followed that described by Davis (*2*). The number of particles per unit volume and the particle size correspond to values reported previously (*2,3*). The amount of unbound pertechnetate was first determined by centrifuging a sample of macroaggregate, separating the supernatant, and counting the supernatant and the precipitated macroaggregates. A more desirable method of determining unbound radioactivity is by paper chromatography. Chromatographic separation of unbound technetium can be done with ascending chromatography using No. 4 Whatman paper in an 85% methanol solution. Under these conditions macroaggregates are found at the origin, and unbound pertechnetate has an R_f value of 56. The pertechnetate peak is sharp and when combined with the activity at the origin (macroaggregate) accounts for over 98% of the total activity in all cases. Free pertechnetate determined in this manner agrees quite well with results obtained by the centrifuging method.

Two factors affect the solubility of oxygen in an aqueous solution. First, the specific solubility of oxygen in water decreases from 39.3 ppm at 25°C to 13.8 ppm at 80°C when the total pressure at the given temperature is 760 mmHg. Secondly, by Henry's Law, the solubility of a gas is zero if its partial pressure in the gaseous phase in contact with the liquid is zero. Zero partial pressure of oxygen can be approached by bubbling nitrogen through the solution, and the condition can be maintained by storage under a nitrogen atmosphere (*1*). Reduced partial pressure of oxygen may also be achieved by storing the solution under a high vacuum.

Each reagent in this study was sterilized and placed in a 30 ml sterile, multiple dose vial. To decrease the initial specific solubility of oxygen, each vial was placed in a beaker of water and heated to about 80° allowing sufficient time for the contents of each vial to come to thermal equilibrium. The contents of each vial were then drawn up in a syringe using a No. 25 needle. All air was displaced from the syringe and the contents injected into a 30 ml sterile, evacuated vial. All evacuated vials were sterile collection vials of the type supplied with the ^{99m}Tc generator by E. R. Squibb and Sons. When preparing macroaggregates, we injected 10–15 ml pertechnetate solution into a fresh, sterile evacuated vial being careful to admit no air into the vial. Each reagent was in turn drawn into a syringe through a No. 25 needle and injected into the vial containing the pertechnetate solution; we were careful to allow no air to enter either the reagent vials or the macro-

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aggregate preparation vial. Subsequently during use, or during the 48-hr testing period, macroaggregate solution was withdrawn without allowing air to enter the vial. During addition of NaOH, the solution turns green (the color of ferrous hydroxide) as reported when the material is prepared under nitrogen (*1*). The green color persists until addition of phenol red.

For the above steps to be effective, it is important that reagent vials and ^{99m}Tc-FHMA storage vials maintain their vacuum. Reagent vials were entered 10 times over a period of 10 days without an apparent loss of vacuum. Macroaggregate storage vials were entered for routine use and for testing over a 2-day period without an apparent loss of vacuum. The vacuum in the vials was not measured either before or after use. A No. 25 needle was used for each entry to minimize the possibility of loss of vacuum.

RESULTS

Macroaggregate samples were divided following preparation, one portion being stored at room temperature and the other being stored under refrigeration. Each sample was tested for unbound technetium immediately after preparation, and the divided portions were tested for unbound technetium at 6, 24, and 48 hr in most cases. Table 1 lists the percentage of unbound technetium in each sample. Figure 1 shows the average unbound technetium as a function of time following preparation. The rates of liberation

TABLE 1. PERCENT FREE PERTECHNETATE IN IRON HYDROXIDE MACROAGGREGATES PREPARED IN VACUUM

Sample No.	Time (hr) after preparation and storage temperature						
	0 hr	6 hr		24 hr		48 hr	
		25°C	4°C	25°C	4°C	25°C	4°C
1	1.8	—	—	—	—	—	—
2	1.9	6.4	—	7.0	—	—	—
3	2.9	9.3	—	12.0	—	13.6	—
4	2.7	8.3	—	9.8	—	13.4	—
5	3.3	8.8	3.5	12.5	4.8	12.4	6.5
6	1.6	2.7	2.0	6.4	2.6	—	—
7	2.0	—	—	7.1	3.4	9.2	3.3
8	2.7	—	—	10.2	5.7	12.0	5.7
9	2.0	3.7	2.4	6.0	3.0	7.4	3.9
10	2.5	5.9	4.8	10.6	5.7	12.7	7.1
11	3.3	—	—	7.5	7.6	10.8	8.5
12	4.6	6.3	5.4	10.3	6.9	10.9	6.9
13	3.2	5.1	3.5	7.5	3.7	11.1	4.8
14	2.3	—	—	6.9	3.4	8.0	4.3
15	4.7	—	—	13.7	6.5	13.8	11.3
16	2.6	4.6	3.2	7.2	3.7	11.0	7.4
17	3.3	6.0	4.2	7.7	5.6	8.5	5.8
18	4.2	7.4	4.3	10.5	6.3	12.6	6.6
19	3.9	7.1	5.6	8.9	6.0	—	—
Average	2.9	6.2	3.9	9.0	5.0	11.1	6.3
s.d.	0.93	1.9	1.2	2.3	1.6	2.2	2.1

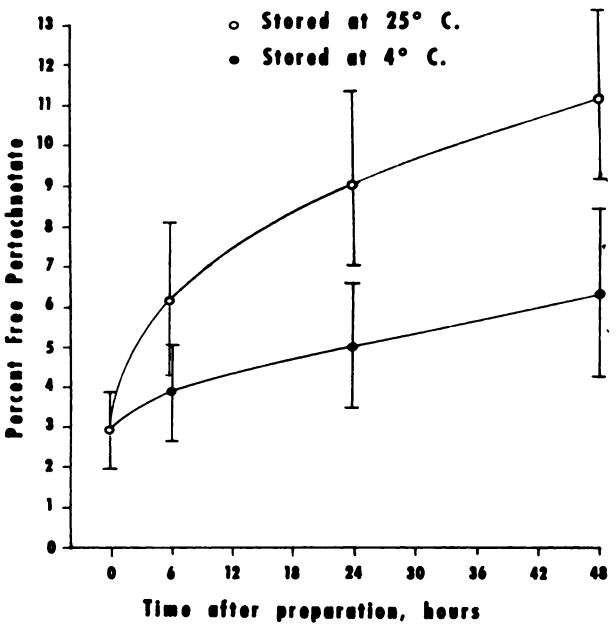

FIG. 1. Average unbound technetium as a function of time following preparation.

of technetium (delta) were calculated from Fig. 1 by treating each graph as two line segments. That is, one line segment extends from preparation time to 6 hr, and the second line segment extends from 6 to 48 hr. During the first 6 hr following preparation of macroaggregates, storage at 4°C reduces delta to 0.17%/hr from 0.55%/hr when stored at 25°C. Subsequent liberation of technetium is at a very low 0.058%/hr when stored at 4°C. A comparable determination of free pertechnetate at 25° and 4°C in an "in air" preparation was not carried out.

Each sample of ^{99m}Tc-FHMA prepared in this study was used in patients for routine lung perfusion studies. Approximately 50 lung scintiphotos have been completed using macroaggregate prepared as discussed above and stored at 4°C. Quality of these lung scintiphotos has been invariably good, showing no signs of thyroid or stomach uptake and no cardiac blood pool activity.

CONCLUSIONS

Preparation of ^{99m}Tc-FHMA under vacuum confirms the previously reported detrimental effect of oxygen upon complete technetium binding. The vacuum technique offers an extremely easy method of accomplishing essentially the same end results obtained by bubbling nitrogen through the solutions. Dissolved oxygen in the pertechnetate solution is not removed from the reaction vial but is reduced to an insignificant level. A loss of vacuum was not observed in any reagent vial; however, this is a possibility. Entry of atmospheric oxygen could be pre-

vented by simply "filling" the reagent vials to atmospheric pressure with nitrogen. Stability of the radioactive tag of ^{99m}Tc-FHMA is considerably improved when stored at 4°C. With negligible increase in labor and preparation time, and no additional equipment requirements, these methods provide ^{99m}Tc-FHMA of uniformly low, free pertechnetate for lung scans and scintiphotos.

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