A New Germanium-68/Gallium-68 Generator

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A germanium-gallium generator producing EDTA-free Ga-68 would permit the synthesis of a broad range of Ga-68 radiopharmaceuticals and thus facilitate the widespread application of positron tomography. We have investigated a solvent-extraction generator system producing the weak complex of Ga-68 with 8-hydroxyquinoline (Ga-68 oxine), free of EDTA. The conditions for optimum Ga-68 yield and minimum Ge-68 breakthrough involve extraction with chloroform from pH 5 buffer containing Ge^{4+} carrier, followed by evaporation to dryness. This produces Ga-68 yields of 70–80%, with Ge-68 breakthrough of <0.003%. The Ga-68 oxine is readily converted to other radiopharmaceuticals such as Ga-68 EDTA, Ga-68 EDTMP, or Ga-68 colloid, and is conveniently delivered dry. The extraction system is simple and amenable to automation, and the low loss rate and 280-day half-life of Ge-68 provide a generator with a long useful lifetime.

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Gallium-68 is of great interest for positron tomography because it emits positrons in high yield and is readily chelated. Furthermore, it is available as a generator product of 280-day Ge-68 and has a convenient 68-min half-life. The current Ga-68 EDTA generators (*1*), however, suffer from several disadvantages. Elution at neutral pH over the long useful life of the generator causes difficulty in maintaining generator sterility. Secondly, Ga-68 can be produced by present generators only as Ga-EDTA. Preparation of radiopharmaceuticals other than Ga-EDTA requires decomposition of the complex and removal of virtually all the EDTA in order for weaker complexing agents to bind successfully with the gallium. The decomposition of Ga-EDTA requires subsequent solvent extraction (*2*), ion exchange (*3*), or pyrolysis (*2*), all of which are tedious and time-consuming when speed is essential. Furthermore, it is doubtful whether these methods can produce radiopharmaceuticals uncontaminated by Ga-EDTA, since even quantities of EDTA as small as 10^{-18} moles in competition with chelates having a stability constant of log K $\simeq$ 10 can lead to Ga-68 preparations that are ~10% Ga-68 EDTA (*4,5*), due to the very high stability constant of Ga-EDTA (log K = 34).

Since this high stability constant is the reason why EDTA elutes Ga-68 from the current column generator, a very different approach seemed necessary to design a generator producing Ga-68 suitable for a wide variety of radiopharmaceuticals.

We have investigated an alternative Ge-68/Ga-68 generator system based on the solvent extraction of the weak chelate of gallium formed with 8-hydroxyquinoline, Ga-68 oxine. The first technique (*6*) used to separate Ga-68 from Ge-68 was in fact solvent extraction of a gallium chelate of acetylacetone into hexane, but the back extraction still employed a dilute solution of EDTA. We have also used the product of this new generator system to produce several Ga-68 radiopharmaceuticals.

MATERIALS AND METHODS

Extractions were performed with standard, 60-ml, Teflon-stopcock separatory funnels, either hand shaken or held stationary and stirred with a motor-driven glass paddle-rod to mix the phases. Unless otherwise noted, oxine dissolved in ethanol to a

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concentration of 1 mg/ml was added directly to the Ga-68 aqueous solution phase before mixing of the phases and extraction. HPLC grade chloroform was used; all other chemicals were standard analytic-reagent grade, used without further purification.

Whatman No. 1PS phase-separatory paper was used to investigate carry-over of the aqueous phase with the organic by filtering the organic phase through the paper and examining it visually for aqueous drops, as well as by counting it the next day to assay for Ge-68. Whatman No. 1 chromatography paper was used for all paper chromatograms, which were made by spotting strips of paper with ~1 μl of solution and developing them by the ascending-solvent method, using chloroform, in ordinary beakers. After development and drying, the chromatograms were cut up with scissors and counted in glass tubes in a well scintillation counter.

Samples containing less than a few microcuries were also counted in a scintillation counter, whereas millicurie quantities were assayed in a radionuclide dose calibrator.

RESULTS

Initial experiments using 5-μCi aliquots of $^{68}GeCl_4$ demonstrated that Ga-68 oxine does extract into chloroform from acetate buffer solutions of Ge-68. The pH dependence is shown in Fig. 1. Extraction does not occur below pH 3.5, and the optimum is about pH 5. Extraction was not attempted above pH 7.0 because of the possible formation of colloidal radiohydroxides. In these experiments the organic phase was passed through Whatman No. 1PS phase-separatory paper after the initial extraction and after a backwash with a second aliquot of aqueous buffer in a separatory funnel to reduce the amount of Ge-68 carried through to the final product. The volume of each phase was 5 ml.

This system gave total extraction yields of Ga-68 oxine approaching 100% at pH 5. About 20% of the Ga-68 oxine remained distributed between the two-phase separatory filter papers and the backwash, and was therefore lost. Also, the amount of Ge-68 lost from the stock solution was approximately 0.04%, a figure unacceptably high. Addition of 10 to 100 mg of NaF, in an attempt to complex and therefore "hold-back" Ge^{4+}, was abandoned because it increased the Ge-68 breakthrough to as high as 1%, possibly due to extraction of hexafluorogermanic acid. Addition of 4 mg carrier Ge^{4+}, making the stock Ge-68 solution 0.01 M in Ge^{4+}, reduced Ge-68 breakthrough to ~0.003%, even when the phase-separatory papers and backwash were dispensed with.

Extraction with CCl_4 as a trial of nonpolar solvent

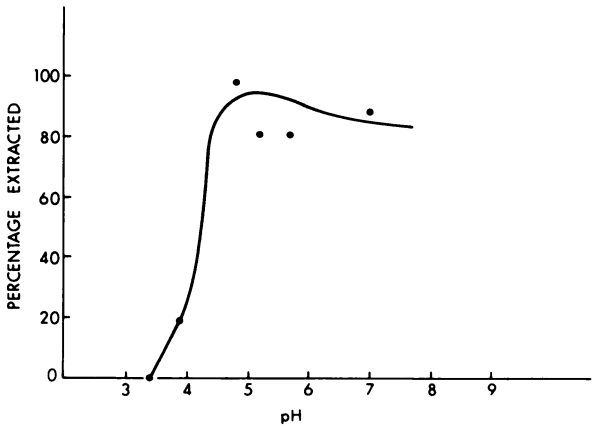

FIG. 1. Dependence of Ga-68 oxine extraction efficiency on pH of aqueous phase.

reduced the Ga-68 yield to ~45%. Extraction with methyl-ethyl ketone resulted in high Ge-68 breakthrough, probably due to the significant solubility of this solvent in water near neutrality. Extraction with CH_2Cl_2 gave the same results as with CCl_3H, although others (7) have reported CH_2Cl_2 to be superior for extracting In-111 oxine.

To prepare a generator containing 3 mCi of Ge-68 it was necessary to neutralize the 0.5 *M* HCl in which the $^{68}GeCl_4$ was supplied*. This resulted in solutions of 0.56 *M* ionic strength, a considerably higher figure than the 0.06 *M* ionic strength of the 5-μCi trial generators. This increased ionic strength decreased the Ga-68 oxine yield from over 90% to about 75%.

Elution of the 3-mCi generator involved addition of 50 μgm of oxine dissolved in 50 μl of ethanol to 5 ml of pH 5 stock Ge-68 solution. The solution was stirred 1 min, after which 5 ml of CCl_3H were added and mixed again, by motorized stirring, for 2 min. The phases were allowed to separate for 1 min and then the chloroform was drawn off manually, care being taken to leave a small volume of chloroform behind to avoid withdrawing any aqueous phase from the flask. The Ga-68 oxine-chloroform solution was then evaporated to dryness using a hot water bath and air stream. The Ga-68 oxine residue was dissolved in 50 μl of ethanol followed by several ml of aqueous solution.

Addition of the oxine solution to the chloroform first, before phase mixing, increased the stirring time required for maximum extraction yield of Ga-68 oxine, as shown in Fig. 2. With this procedure the activity of Ga-68 oxine produced by the generator can be controlled conveniently by varying the stirring time.

The actual performance of a 3-mCi generator is

shown in Fig. 3. The generator was eluted at intervals over a two month period (adding oxine solution directly to the Ge-68 solution) and the yields obtained were plotted against time. The fact that this plot matches the 280-day half-life of Ge-68, within the normal variation of the generator yield, confirms the generator system's longevity.

Radiopharmaceutical preparation. The Ga-68 oxine residue, dissolved with a small amount of ethanol and diluted with saline, will directly label separated blood components such as erythrocytes, leukocytes, and platelets (*8*). The labeling of concentrated red cells, for example, is essentially complete in 10 min, as shown in Fig. 4. The labeling yields were obtained using red cells at the same concentration as in whole blood; if the red cells are diluted by a factor of more than five, the yield drops drastically. Similar techniques have been used in our laboratory to label platelets with Ga-68 for imaging thrombi; the labeling yields were more uniform than in work previously described (*9*).

The stability constants of Ga-oxine are less than the stability constants of Ga with chelates of interest for in vivo use (*4,5*). This suggested that the addition of stronger chelating agents should lead to the rapid displacement of oxine to form other gallium chelates. We have shown this to be the case chromatographically for the formation of Ga-68 EDTA from Ga-68 oxine. When a sample of Ga-68 oxine was eluted with chloroform, the Ga-68 oxine moved with the organic solvent front. When 1.5 ml of the same Ga-68 oxine-saline solution was mixed with a saline solution of disodium EDTA (15 mg in 1.5 ml), and incubated 5 min before spotting on chromatography paper and elution with chloroform, virtually all the Ga-68 remained at the origin, as expected for Ga-EDTA.

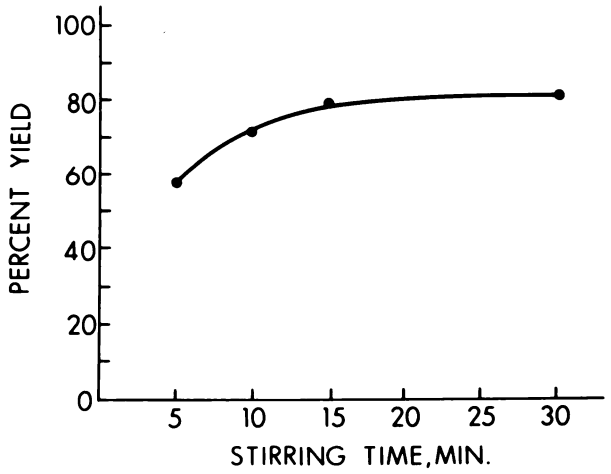

FIG. 2. Dependence of Ga-68 extraction efficiency on mixing time when oxine is initially present in the chloroform phase only.

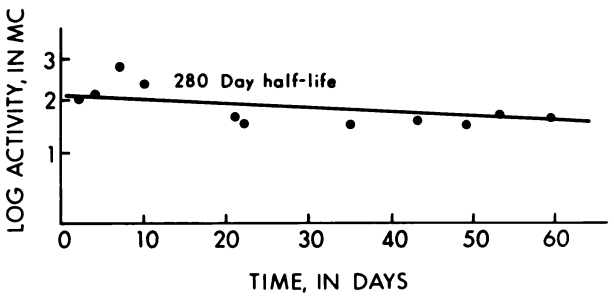

FIG. 3. Actual performance of a scaled-up Ga-68 oxine solvent-extraction generator.

Similarly, addition of Ga-68 oxine solution to DTPA-labeled albumin has been demonstrated chromatographically in our laboratory (*10*) to lead to Ga-68-labeled protein. Thus, the use of an intermediary chelating agent bound to a protein makes it possible to label that protein with Ga-68 from this generator system.

We have also made Ga-68 ethylenediamine tetramethylene phosphonic acid (Ga-68 EDTMP) by addition of Ga-68 oxine saline solution to a saline solution of EDTMP, followed by incubation for several minutes and neutralization to pH 7. Using this preparation we have obtained excellent bone uptake in rats and rabbits, but chromatographic evidence suggests possible impurities in the EDTMP. We have produced a Ga-68 colloid by the addition of 50 μgm $FeCl_3$ in 50 μl of 1 *M* HCl solution to 50 μl of ethanol containing the Ga-68 oxine residue, followed by 1.5 ml pH 7.5 phosphate buffer.

DISCUSSION

This new Ga-68/Ge-68 generator system has several advantages over the Ga-68 EDTA generator. Elution with chloroform simplifies generator sterility, and dry delivery of the Ga-68 oxine product provides great flexibility in radiopharmaceutical preparation, especially with respect to concentration. Finally, delivery of EDTA-free Ga-68 greatly simplifies the preparation of a wide variety of radiopharmaceuticals. The presence of 50 μg of oxine in all preparations should pose no problems, since the oxine will be displaced, essentially quantitatively, in all cases and since oxine at this level has been shown to be safe for in vivo use (*11–14*).

For purposes of comparison, the original paper (*1*) describing the Ga-68 EDTA column generator indicates a yield of 70%, with a Ge-68 breakthrough of 0.0003%. In our experience with a 25-mCi Ga-68 EDTA commercial generator, the yield drops to about 45% as the generator ages, whereas the new Ga-68 oxine generator has a consistent yield of about 75%. The Ge-68 breakthrough for either gen-

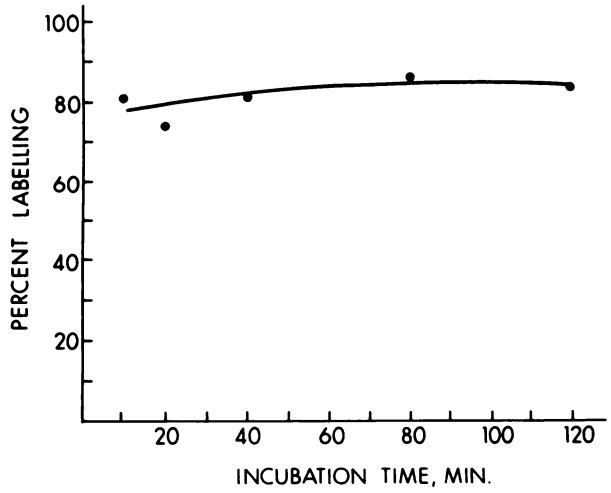

FIG. 4. Demonstration that red-cell labeling is essentially complete in 10 min using Ga-68 oxine.

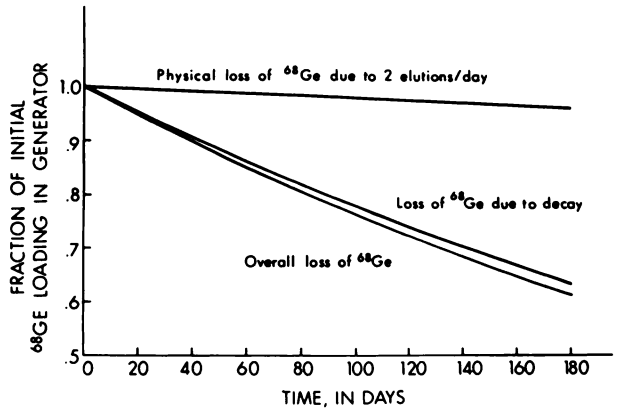

FIG. 5. Demonstration that Ge-68 breakthrough losses of 0.01% per elution (an upper limit) are insignificant compared with Ge-68 decay loss, assuming two elutions per day.

erator in our experience is approximately 0.003%. Since Ge carrier is present in the Ga-68 oxine generator, about 0.12 μg of stable Ge is present in the eluant, whereas the amount of radiogermanium in the eluant of a Ga-68 EDTA generator is less than 1 ng.

Generator longevity should pose no problem, as shown in Fig. 5. The upper curve shows the rate of loss of Ge-68 due to breakthrough, assuming two elutions per day and 0.01% loss per elution (an upper limit). The middle curve represents loss of Ge-68 due to decay only, while the bottom curve represents total Ge-68 loss. Thus, the breakthrough losses are considerably less significant than the decay loss. As described above, our experience over time with a 3 mCi generator has confirmed this prediction.

The factors affecting the generator yield are readily interpreted chemically. The diminution of yield with higher ionic strength is probably due to a stabilization of Ga^{3+} in the aqueous phase by other ionic species, thus shifting the partition coefficient. The increased mixing time required to obtain maximum yield when oxine is added to the chloroform phase first is probably due to the much greater solubility of oxine in chloroform than in aqueous solution. When the oxine is added to the aqueous phase first, it rapidly forms Ga-68 oxine, which is then subject to extraction. When the oxine is originally present in the chloroform phase only, it must enter the aqueous phase, complex the Ga-68, and be re-extracted into the chloroform. This process is undoubtedly slower and more dependent upon surface contact between the phases, and hence requires a longer mixing time to obtain maximum yields. The lowering of yield observed when CCl_4 was used as the extractant may reflect the preference of Ga-oxine for a solvent that is not completely nonpolar.

In about a year of manual operation of the generator, no accidents have occurred. Automation of solvent-extraction generators has been successfully applied to other systems (*15*). We anticipate automation of this Ga-68/Ge-68 generator system using optical detection of the phase interface to control solenoid valves to effect the phase separation, as well as mechanical stirring for phase mixing. In such a system, of course, great care must be taken to ensure that entrapment of the aqueous phase does not occur in the phase separation.

The similarity in extraction conditions for Ga-oxine and In-oxine (*16*) is not surprising, since the elements are chemical homologs. Thus it might also be expected that procedures for producing indium-tagged radiopharmaceuticals would be applicable to Ga. This is indeed the case for Ga-68 EDTA, Ga-68 EDTMP (*17,18*), Ga-68 colloid (*19,20*), and Ga-68-tagged platelets.

FOOTNOTE

* New England Nuclear, Boston, Mass.

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