**PRELIMINARY NOTE**

# A New Cd-115 → In-115m Radionuclide Generator

Gary J. Ehrhardt, Wynn Volkert, William F. Goeckeler, and Donald N. Kapsch

*University of Missouri Research Reactor, University of Missouri-Columbia, Department of Chemistry, and University of Missouri Health Sciences Center, Columbia, Missouri*

**A new column-type Cd-115 → In-115m generator has been developed by adsorbing $CdI_4^{-2}$ on an anion-exchange resin and eluting the In-115m with 0.05 *M* HCl. The In-115m yield of the prototype column is 90% in a volume of 3 ml, with Cd-115 breakthrough of less than $3 \times 10^{-4}$%. Over thirty generators with up to 40 mCi of activity have been produced using components of a commercial Mo-99 → Tc-99m generator system; they behaved like the prototype. In-115m oxine prepared from these generators has been used to label canine platelets and to image an induced canine thrombus in vivo.**

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Recent interest has focused on indium radioisotopes in nuclear medicine, primarily In-111 because of that nuclide's ability to label cellular blood components when complexed with agents such as 8-hydroxyquinoline, acetylacetonate or tropolone (*1*). We have investigated the generator production of In-115m, a radionuclide that seems promising for medical imaging due to its half-life of 4.5 hr, which is comparable to that of Tc-99m and more convenient than the shorter-lived, 99.5-min In-113m. Secondly, In-115m can be generator-produced from inexpensive, reactor-irradiated Cd-114, whereas In-111 must be produced directly by cyclotron irradiation. And, unlike Tc-99m, chemical reduction by stannous ion or other means is unnecessary for complex formation with indium, which has only one prominent oxidation state in aqueous solution. Thus the chemistry of indium is simpler than that of technetium, although perhaps less versatile. Finally, as will be shown, an acceptable Cd-115 → In-115m column-type generator is easily produced from commonly available materials and can be used to label platelets.

**Generator system.** The Cd-115 → In-115m generator system is illustrated below (*2*).

$$^{115}\text{Cd}\ (53.4\ \text{hr}) \xrightarrow{\beta^-} {}^{115m}\text{In}\ (4.49\ \text{hr})$$

$$\downarrow \gamma$$

$$^{115}\text{In}\ (5.1 \times 10^{14}\ \text{yr})$$

The parent Cd-115 is conveniently and easily produced by neutron irradiation of enriched Cd-114 target material, and decays by beta emission to In-115m. The decay characteristics of In-115m are shown in Table 1 (*3*).

The presence of particulate radiation from In-115m raises the question of radiation dosage. Table 2 lists the splenic and total-body doses from 300 μCi of platelets labeled with In-111, In-113m, and In-115m, uniformly distributed in the spleen, and 700 μCi of the same isotopes uniformly distributed in the total body as blood-pool platelets (*4*). It is evident that even considering the one-fourth photon abundance of In-115m as compared with In-111, the dose per photon from In-115m is less than that from In-111.

## MATERIALS AND METHODS

Initial work was performed using 10 μCi amounts of Cd-115 produced by short neutron irradiations of 10 mg of 99.999% pure Cd metal. Larger yields were prepared by longer bombardments of 10–20 mg of 98.6% enriched $^{114}$CdO (cost: $0.45 per mg Cd) obtained from Oak Ridge. A typical irradiation at $10^{14}$ neutrons $cm^{-2}$ $sec^{-1}$ lasted 2–3 days, and yielded 40–60 mCi of Cd-115. Both types of targets were dissolved in hot, concentrated nitric acid and boiled to dryness several times with concentrated hydrochloric acid. The resulting cadmium chlo-

**TABLE 1. In-115m DECAY CHARACTERISTICS***

| Radiation | Energy | % Abundance |
|---|---|---|
| Gamma | 336 keV | 45.9 |
| Conversion electron | ~300 keV | 49.1 |
| Beta particle | 840 keV | 5.0 |

* Ref. 3.

**TABLE 2. RELATIVE DOSIMETRY FOR INDIUM ISOTOPES**

| Isotope | $\bar{D}$ (spleen → spleen) + $\bar{D}$ (total body → spleen) (mrem) | $\bar{D}$(total body → t.b.) + $\bar{D}$(spleen → t.b.) (mrem) |
|---|---|---|
| In-111 | 27,000 | 590 |
| In-113m | 1,440 | 17 |
| In-115m | 4,700 | 49 |

ride residue was dissolved in 10 ml deionized water to which was added a two-fold excess of potassium iodide crystals (8 mmole $I^-$/mmole $Cd^{+2}$) and allowed to stand 10 min to form the complex $CdI_4^{-2}$. All chemicals were of analytical reagent grade.

Prototype column generators were prepared using glass Econo-columns,* 0.7 cm i.d. and packed to a height of 10 cm with untreated AG1X8 anion exchange resin,* 200–400 mesh, received in the chloride form. The loading solution of cadmium iodide in deionized water at pH $\simeq$ 6 was poured into a reservoir on the top of the column and drawn through at a rate of 1–2 ml/sec into an evacuated vial using a three-way Luer stopcock and needle placed on the end of the column. This was followed by 20 ml 0.05 *M* HCl eluant to wash out any unbound cadmium.

Elution of the prototype generator was accomplished by adding 10 ml of 0.05 *M* HCl eluant to the reservoir and drawing the solution through the column using an evacuated serum vial at 1–2 ml/sec flow rate. Enough solution was left in the column to keep the resin wetted with eluant at all times.

Loading efficiencies of Cd-115 and elution yields of In-115m were obtained by comparison of samples with an aliquot of stock Cd-115→In-115m solution using either a NaI(Tl) well counter and 1,024 channel analyzer or an efficiency-calibrated Ge(Li) detector and analysis system. Separation of the 336 keV peak of In-115m from the 527 keV peak of Cd-115 was easily accomplished with either instrument. Counting of the latter peak compared with a standard after the In-115m had decayed sufficiently gave data on Cd-115 breakthrough. The same system was used to analyze for radionuclidic impurities in the eluate.

Production-style generators were produced from new components and shells of Mo-99 → Tc-99m generators.† The standard column, 0.7 cm i.d. by 4 cm long was packed with AG1X8 anion-exchange resin and washed with three 5-ml portions of ethanol, followed by similar washes with 0.05 *M* HCl, and finally with deionized water. The cadmium-115 iodide solution was passed through the column using a syringe and capillary tubing, after which the column was connected to the elution reservoir and valve assembly. The generator was then assembled and 50–100 ml of 0.05 *M* HCl eluant were drawn in the normal manner for that generator type to remove any unbound Cd-115.

Labeling of equine and canine platelets was accomplished using 50 ml of whole blood and a modified version of the method of Thakur and Welch (*5*), in which chloroform extraction of the (8-hydroxyquinoline) oxine-indium complex is not necessary (*6*). Specifically, 50 $\mu$gm oxine in 50 $\mu$l absolute ethanol were added to approximately 0.5 ml of $^{115m}InCl_3$ generator eluate, followed by 4 ml of modified ACD:NS solution (one part acid citrate dextrose to seven parts 0.9% saline). The product was adjusted to pH 6.5 with 1 *N* NaOH and used as the labeling solution in the normal manner. Platelet labeling yields were determined with a dose calibrator. Platelet aggregation was induced by addition of either collagen or ADP, and the aggregability measured with an aggregometer by a method similar to that described by Thakur et al. (*7*). Labeled platelets and unlabeled platelets from the same animal were compared.

A canine venous thrombus was induced in the neck of an adult mongrel dog by irritation of the endothelium with a catheter. Imaging of In-115m-labeled autologous platelets was performed 2 hr after injection using a conventional scintillation camera and a medium-energy collimator.

## RESULTS

An initial attempt was made to reproduce a reported (*8*) Cd-115 → In-115m separation by column chromatography using zirconium phosphate inorganic cation-exchange media from two sources. This failed, as did attempts to use a hydrated antimony pentoxide cation-exchange medium.

We then considered forming an anionic Cd-halide complex that might adhere to an anion-exchange column. Previous work (*9*) involved use of the $CdCl_4^{-2}$ complex adsorbed on an anion-exchange resin, but did not seem to provide acceptably low Cd-115 breakthrough. Stability constant data (*10*) suggested the $CdI_4^{-2}$ complex, which is considerably more stable than the $CdCl_4^{-2}$ anionic complex. A cadmium bromide anionic complex also exists, but is intermediate in stability between the chloride and iodide complexes.

Prototype* generators exhibited a loading efficiency for Cd-115 of greater than 99%, and In-115m yields of ~90% based on the activity of In-115m available on the column. Using 10 mCi prototype columns, 10 cm high, breakthrough of Cd-115 was below our detection limit of $1 \times 10^{-4}$%, with no significant radionuclidic impurities in the eluate. Breakthrough of Cd-115 was not detected with these generators over a time span of eight days, although apparent radiation damage to the resin was observed after several days. The top 1.5 cm of the aging resin bed exhibited a chocolate-brown discoloration that roughly coincided with the position of the activity on the column as ascertained with a hand monitor. When a portion of the discolored resin was shaken with chloroform, no purple extract appeared, suggesting that the color was not due to free iodine. Flow rates during both loading and elution were not critical; lower rates than the 1-2 ml/sec achieved using evacuated vials did not improve generator performance.

An elution curve of a prototype generator obtained by fractional elution in 1-ml increments showed that the bulk of the In-115m was found in the second ml, with 90% of the yield eluted after 3 ml and 95% after 4 ml.

Production generators in commercial shells† gave performance similar to that of the prototype generators except with respect to Cd-115 breakthrough. Figure 1 shows breakthrough of Cd-115 from a typical 40-mCi production generator over a period of two weeks. At no time did the breakthrough exceed $4 \times 10^{-3}$% of the Cd-115 activity on the column; indeed, the fractional breakthrough decreased with age of the generator. The yield of In-115m, however, remained constant and high with time, as demonstrated by Fig. 2 for the same generator. Concentrations of In-115m as high as 20 mCi/ml were obtained.

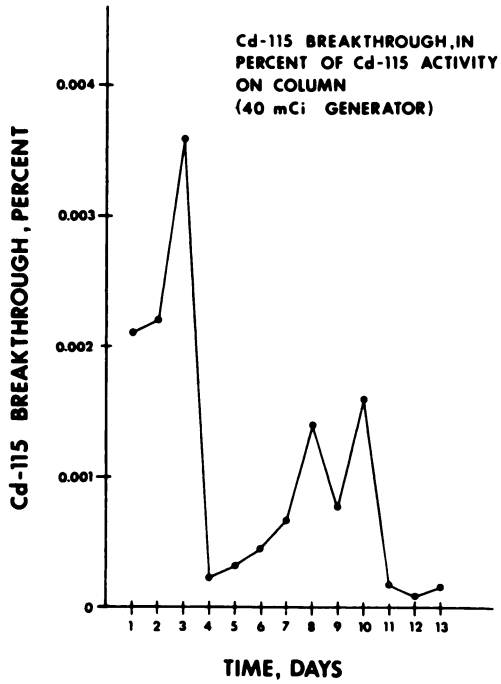


FIG. 1. Amount of Cd-115 eluted with In-115m in a 10 ml volume daily against time in days, for generator of 40 mCi initial activity. Amount of Cd-115 is expressed as percentage of the Cd-115 remaining on column at time of elution.

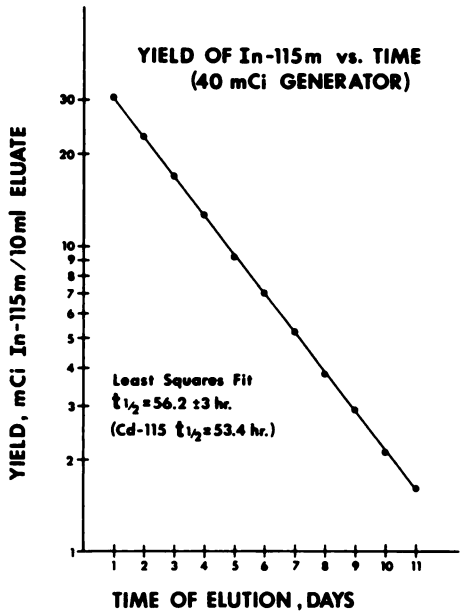


FIG. 2. Yield of In-115m (mCi) vs. time of elution in days for production generator of ~40 mCi activity produced using commercial Mo-99 → Tc-99m generator components.

Labeling efficiency for equine and canine platelets using varying amounts of In-115m eluate up to several milliliters was 70–75%. Labeled canine platelets were observed to function satisfactorily in an aggregometer.

Figure 3 demonstrates visualization of a canine venous thrombus at the site of induced vascular damage in the neck of a dog using autologous platelets labeled with In-115m oxine, thus furnishing evidence of the in vivo viability of the labeled platelets.

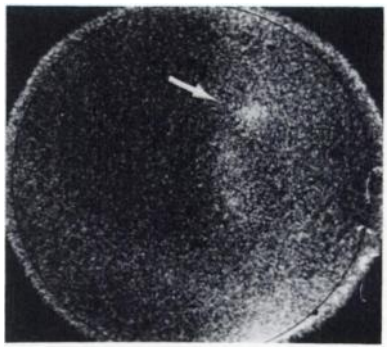

FIG. 3. Scintiphoto of head, neck, and right foreleg of adult mongrel dog with induced venous thrombus (arrow) in the neck, visualized using In-115m-labeled autologous platelets. Some tailing of thrombus beneath its head may also be seen.

## DISCUSSION

The question may be raised as to whether chloride ion in the eluting solution might not convert the $CdI_4^{-2}$ on the column to the corresponding chloro complex, especially in view of the relatively labile nature of these cadmium halide complexes. It must be remembered, however, that such a substitution reaction depends upon the lability of iodide in the resin-adsorbed complex, which could differ from the lability of iodide in a solution of the free $CdI_4^{-2}$ complex. In any event, our purpose was to produce a usable, practical In-115m generator rather than to explore the column chromatography behavior of cadmium halide complexes. The use of the cadmium iodide analog rather than the cadmium chloride complex seems prudent in any case in view of the greater stability of $CdI_4^{-2}$ and the known affinity of the resin for polarizable, "soft" anions (*11*).

The success achieved with platelet labeling shows that this generator design produces an eluate minimal in metal impurities that inhibit indium-oxine complex formation, indicating that the eluate is well suited for labeling cellular blood components. In work to be reported later, we have also found that preparations of In-115m from this generator do not exhibit acute toxicity in mice, although further work is necessary to establish complete safety of the product.

Imaging with In-115m-labeled blood cells may be used in human disease states in which significant platelet or white cell accumulation occurs at sites of thrombi, vascular lesions, or inflammation within a time span compatible with its 4.5 hr half-life. In contrast, the half-life of In-113m (99.5 min) is inconveniently short and its gamma energy higher than is desirable (393 keV). The current radionuclide used for platelet labeling, In-111, persists in the body several times longer than In-115m, and thus may interfere with subsequent radionuclide studies. In addition, generator-produced In-115m is potentially much cheaper per mCi than the In-111 in widespread use. Lastly, although not optimal for use with current Anger cameras, the higher gamma energy of In-115m (336 keV) is still suitable for collimators designed for I-131 (364 keV), and may be useful for dual-nuclide studies where Tc-99m agents have already been administered. The higher-energy photons of In-115m, used as a blood-pool label by binding ionic indium with transferrin, may also be useful for better quantitation of cardiac stroke volume in cases where absorption of Tc-99m gammas from a large or deeply seated heart may produce errors.

One final consideration concerns the question of radiation dosage to labeled cells from low-energy, high LET Auger and conversion electrons. Using the methods and assumptions of Bassano and McAfee (*12*) and data from MIRD Pamphlet No. 10 (*13*), we find that the internal dosages to platelets recovered from 30 ml whole blood and labeled with 1 mCi each of In-111 and In-115m are 12,900 rads and 600 rads, respectively. Since the photon yield ratio of In-111 to In-115m is 4.67 (*2*), the internal radiation dose to labeled platelets on a per photon basis from In-115m is less than that of In-111 by nearly a factor of five.

## CONCLUSION

Indium-115m can be made available conveniently, in high yield, and in high purity from a simple Cd-115 → In-115m column generator system. This radionuclide has potential for a variety of imaging and dual-emitter studies.

## FOOTNOTES

* Bio-Rad, 32nd & Griffin Avenue, Richmond, CA 94804.
† Mallinckrodt Ultratechnekow.

## ACKNOWLEDGMENTS

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