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Abstract: The theme of this session was labelling molecules with short-lived radioisotopes. Descriptions of the preparation of ligands of both small and large molecules were presented.

Keywords: Lymphoscintigraphy; Mannose receptors; PET; Antibodies

RECEPTOR TARGETED LYMPHOSCINTIGRAPHY USING DEXTRAN DERIVATIVES LABELLED WITH ^{99M}TC: IN VIVO PRELIMINARY BIOLOGICAL EVALUATION

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Abstract: The radiolabelling of mannosyl-dextran derivatives with ^{99m}Tc resulting in a high purity and stability radiolabelled conjugate, suitable for sentinel node detection with low distal lymph accumulation, as well as their in vivo biological evaluation are the proposed aims of this study.

Different radiolabelling strategies, including novel ^{99m}Tc cores, were evaluated in order to select and optimize the most efficient and specific one. The analysis of radiolabeled conjugates using TLC and HPLC was performed; the RCPs of the probes were ranged between 93-99%. The biological evaluation (ex-vivo biodistribution and specific uptake) was performed in Wistar rats at 15, 30 and 60 min post injection. The biological data shows a rapid and highly specific sentinel node accumulation, up to 7.5% ID, and a very good sentinel node extraction in respect with the second node in the chain up to 83% at 1h p.i.

Keywords: lymphoscintigraphy; lymphnode detection; mannose receptors

Introduction: Use of ^{99m}Tc in studies for developing cancer specific radiopharmaceuticals offers the possibility of cost effective applications[1]. Numerous radiopharmaceuticals are presently utilized for sentinel node detection: ^{99m}Tc-sulfur colloid, filtered ^{99m}Tc-sulfur colloid and different microcolloids of ^{99m}Tc-labelled albumin, but none of these agents have ideal properties regarding the selective accumulation in sentinel node, without uptake in the distal lymph node. A lymphoscintigraphy agent requires a high density of receptor substrate sites to achieve a receptor affinity required for proper sentinel node detection[2,3]. The solution is a receptor-binding radiopharmaceutical which can be synthesized with high specific activities, compatible with typical target tissue receptor densities. The pre-operative lymphoscintigraphy is essential and leads to the identification of the lymph node chains that may present metastatic disease[1]. Intra-operative gamma detection permits easier localization of the sentinel lymph node in a less aggressive dissection and complements blue dye mapping[1,2]. It is desirable to make intra-operative search of the same radiopharmaceutical injected for pre-operative lymphoscintigraphic exam[3]. It has been established that there is a narrow relationship between the size of a radiopharmaceutical and its properties of diffusion in the lymph node, the bigger the particle, lesser the diffusion and higher the retention in the lymph node[4,5]. Mannosylated macromolecules labeled with ^{99m}Tc[6-9] were investigated for targeted lymphoscintigraphy, and better uptake in the sentinel lymph node is expected due to their specific binding to mannose receptors expressed on lymph node macrophages[5].

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Materials and Methods: *Synthesis of mannosyl-dextran derivatives*

Mannosyl-dextran derivatives were synthesised in different laboratories and evaluated in our lab. Probe labels are: DC1, DC2, DCAM, DCCM. The probe differ by molecular weight and functional groups appended to dextran backbone for radiolabeling with ^{99m}Tc. The mean diameter of each conjugate was measured by dynamic light scattering (Zetasizer Nanoseries ZS90-Malvern, UK). The probes were assayed at 0,32 mg/mL in 0.9% sodium chloride. The equipment was calibrated using latex particle standards. The mean hydrodynamic diameters were determined by the analyzer software from volume distribution data.

Radiolabelling of conjugates using ^{99m}Tc tricarbonyl core

The $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor was prepared according to the product leafet, by adding $^{99m}TcO_4^-$ generator eluent (1 ml, 0.74-1.85 GBq) to an IsoLink™ kit vial (Covidien, The Netherlands) containing sterile lyophilized formulation, heating at 100°C for 20 min. in a water bath. After cooling, 120 μL 0.1 M HCl was added to pH 10.5. To each probe vial containing 400 μg dextran derived compound, 250 μL of the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor and 250-500 μL saline were added and probes were incubated at 70°C for 20 min, in a water bath. After cooling the radiochemical purity of the products was analyzed by TLC and HPLC.

Radiolabelling of conjugates using ^{99m}Tc nitrido core

Succinic dihydrazide (SDH) (0.1 mg) and SnCl2 (0.1 mg in 0.1 mL of saline) were placed in a vial, which was then flushed with nitrogen for 2 min and sealed. After addition of 0.9 mL of $[^{99m}TcO4]^-$, the vial was left at room temperature for 15 min. Ligands DCCM (0.1 mg dissolved in 0.5 mL of saline) and PCN (0.5 mg dissolved in a saline solution containing 2.0 mg of γ-cycOH after short sonication) were successively added to the same reaction vial and the resulting mixture was heated for 15 min at 80°C.

Analysis: The radiochemical purity of ^{99m}Tc radiolabelled dextran derivatives was determined by ascending instant thin-layer chromatography (ITLC) with silicagel-coated fiberglass sheets 20 cm length (Polygram SIL G, Macherey-Nagel, Germany) using either acetonitrile, physiologic saline (0.9% NaCl) or acidified methanol (0.1% TFA) as the mobile phase. 5 μL samples were spotted on TLC strips, and analyzed as described. Radioactivity associated with the chromatographed ITLC strips was determined using a radiation scanner (mini Gita TLC, Raytest). The radiochemical purity of the probes was determined with GitaStar TLC software. The radioactive contaminants were identified as reduced/hydrolyzed ^{99m}Tc, free $^{99m}TcO_4^-$ and $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursors.

Alternatively, HPLC (Shimadzu HPLC System) with radiation detection (Berthold) was employed for radiochemical characterization of the ^{99m}Tc radiolabelled dextran derivatives using a Nucleosil C-18 reversed-phase column, 5 μm, 250 mm × 4.6 mm (Supelco Inc.), with the following gradients:

a. A, (0.1%-TFA in H2O), B, (0.1%-TFA in MeOH): 0 min, B, 10%, 0-28 min linear to 90:10 MeOH:water; 28-35 min 90:10 water: MeOH.
b. A, (0.1%-TFA in H2O), B, (0.1%-TFA in CH3CN): 0 min, B, 0%; 0–15 min, B, 100%; 1525 min, B, 0%.

^{99m}Tc-labelled rhenium sulphide (Nanocis) preparation and analysis

The radiopharmaceuticals were prepared according to the instructions. The labelling was performed using ^{99}Mo/^{99m}Tc generator (4.3 GBq, Ultratechnekow, Mallinckrodt Medical, The Netherlands). 2 mL $Na^{99m}TcO_4$ 0.2-0.8 GBq was used for the labelling. RCP was tested after labeling by PC (Whatman 1), mobile phase MEK. The RCP of the radiocolloid was 97%.

Animal studies– experimental model

Young male Wistar rats (*rattus norvegicus* albinos variety, rodentia, mammalia), 200-250 g in size, were used and kept in cages under ambiental temperature and humidity, receiving commercial ration and water *ad libitum*. The animals were anesthetized with a mixture (0.2 mL/animal) containing 0.15 mL ketamine 10% and 0.05 mL acepromazine (Calmivet), injection using an insuline syringe and needle in the peritoneal cavity. This concentration assured about 60 min. of anesthesia and if necessary, additional doses were injected.

Preliminary examinations: one rat was injected with methylene blue solution, paraarticulary, 2 × 0.05 mL, laterally and medially near the knee joint of the right rear foot. At 10-15 min postinjection the poplitear lymph node and the inguinal lymph node was clearly observed blue painted in the exterminated animal.

Biodistribution procedure

Rats (n = 3) were injected subcutaneously in the rear right foot pad with 0.1 mL (10 MBq)/animal ^{99m}Tc-dextran-derived conjugate. At 45 min pi, rats were injected with methylene blue solution, paraarticulary, 2 x 0.05 mL, laterally and medially near the knee joint of the right rear foot. Rats were sacrificed at 1 h pi. The popliteal lymph node, the inguinal lymph node, the right rear foot pad, blood and liver samples were prelevated and placed into plastic tubes. All probes were weighed and counted (gamma counter Raytest). The percentage of the injected dose per g organ (%ID/g) and the percentage of injected dose per organ (%ID) were calculated as medium values from individual measurements. The popliteal lymph node extraction (E) was calculated as:

$$E1(\%) = \frac{\%ID_{POPLITEAL} - \%ID_{ILIAC}}{\%ID_{POPLITEAL}} \times 100$$

In vivo uptake testing by autoradiography

Animal models were injected in the foot pad, under anesthesia, with 100 μL (10-15 MBq) of ^{99m}Tc-radiolabelled dextran derived compounds. Groups of 2 rats were imaged at 5, 10, 30 and 60 minutes pi. For autoradiography, the animals were simultaneously placed under anesthesia on a storage phosphor screen MP (Perkin Elmer Life Sciences, USA) and left there in the dark for 15 to 60 sec., depending on activity. In addition, an optical photograph of the animals was taken. The autoradiographs were developed using a Cyclone Phosphor Imager (Perkin Elmer Life Sciences) and analyzed using Optiquant software (Perkin Elmer Life Sciences). The position of the radioactive areas was matched by overlaying autoradiographs and photographs.

Results and discussions: Synthesis of mannosyl-dextran derivatives

The obtained radiochemical purities (RCP) of the dextran derived compounds were higher than 90% (Table 1). The retention times for each probes in the solvent systems a and b are presented in Table 2.

Table 1. RCP of dextran-derived compounds determined by ITLC and HPLC

QC	DC1	DC2	DCAM	DCCM
ITLC	93.5%	93.1%	94.9%	77%
HPLC	90.8%	98.9%	93.0%	75%

Table 2. Retention times of chemical species determined by HPLC

Solvent system	DC1	DC2	DCAM	DCCM	TcO_4	$Tc(CO)_3(H_2O)_3$
a	14.2	14.24	17.5	—	5.2	10
b	10.1	10.16	10.28	13.8	7.37	12.7

Autoradiography studies

After successful labeling of dextran derived compounds with ^{99m}Tc we explored their imaging efficacy in *in vivo* systems. The semiquantitative autoradiographs (Figures 1-3) were analyzed and the resuls are summarized in Table 3. The regions of interest were selected, corresponding to radioactive areas, the luminiscence units per mm^2 are reported as well as the percent of sum of regions. The autoradiographs show a high concentration of dextran derived compound DC1 and DC2 in sentinel (popliteal) lymph node and a good, but not stable, accumulation of DCAM in the target. There is also a semnificative liver uptake of DCAM starting at 10 min p.i. and DC1 at 60 min p.i. The autoradiographs of DCCM show an unspecific uptake and large radioactive areas corresponding to the whole body of the animals. The Nanocis standard autoradiography shows very good uptake in the target lymph node, a "clean" liver and a semnificative uptake in the bladder at 60 min p.i.

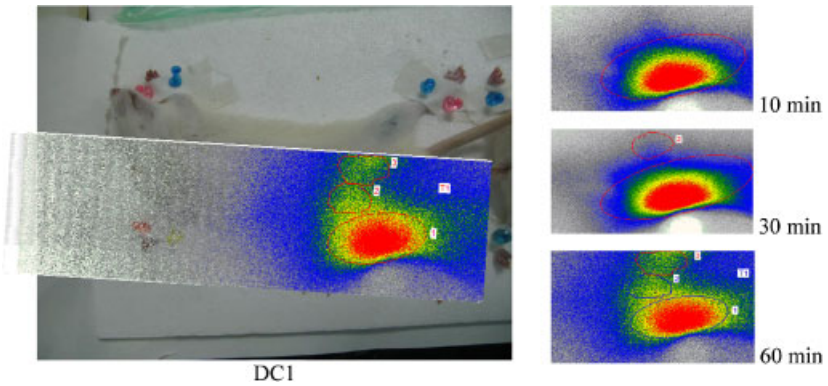

Figure 1. Autoradiographic images (5, 10, 30 and 60 min) and photo of a rat injected with ^{99m}Tc-DC1.

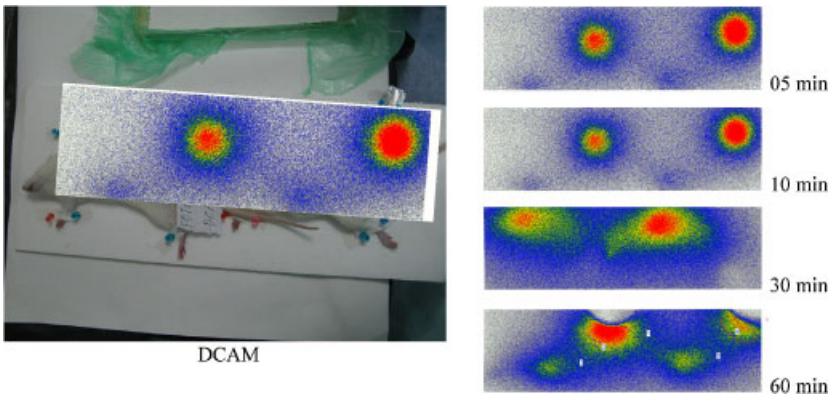

Figure 2. Autoradiographic images (5, 10, 30 and 60 min) and photo of two rats injected with ^{99m}Tc-DCAM.

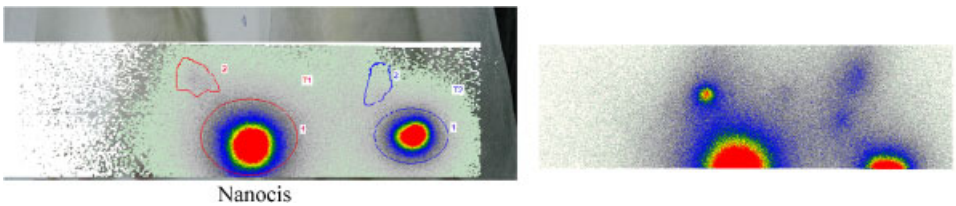

Figure 3. Autoradiographic images (30 and 60 min) and photo of two rats injected with ^{99m}Tc-Nanocis.

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Biodistribution studies

The biodistribution studies confirm the preliminary in vivo evaluation and gave more accurate quantitative uptake data. The results of the study are graphically presented in Figure 4. The ID/g for prelevated organs: blood, liver, sentinel (popliteal) lymph node and second (iliac) lymph node and the injection site weres represented for comparative evaluation. Very good specific uptake of DC1 (7.52% ID) and DC2 (4.52% ID) in the popliteal lymph node was observed, corresponding to 173, 106, 109 and 265 % ID/g organ. The popliteal node extractions are presented in Table 4. Specific uptake of radiolabelled derivatives in the first lymph node was poor in the case of DCCM and very good in the case of DC1 (83.6%).

Table 3. Autoradiographs analysis

		5 min (DLU/mm^2)	region (%)	10 min (DLU/mm^2)	region (%)	30 min (DLU/mm^2)	region (%)	60 min (DLU/mm^2)	region (%)
DC1	SLN	65.222, 4	100, 0	108.701, 8	100.0	247.478, 7	96.1	80.199, 6	73.2
	2 LN	—		—		86.539, 5	3.9	55.892, 3	12.8
	Liver	—		—		—		51.495, 7	14.0
DCAM	SLN	12.329, 6	89, 9	19.319, 1	82.1	37.979, 8	83.6	32.409, 6	63.7
	2 LN	—		—		—		20.507, 6	9.6
	Liver	3.971, 9, 10, 1		5.248, 7	17.9	26.764, 2	16.4	18.268, 6	26
Nanocis	SLN	Not taken		188.856, 0	97.4	40.349, 8	86.5	30.525, 4	57.8
	2 LN			27.071, 2	2.6	—		—	
	Bladder			—		20.516, 2	13.5	13.939, 9	42.2

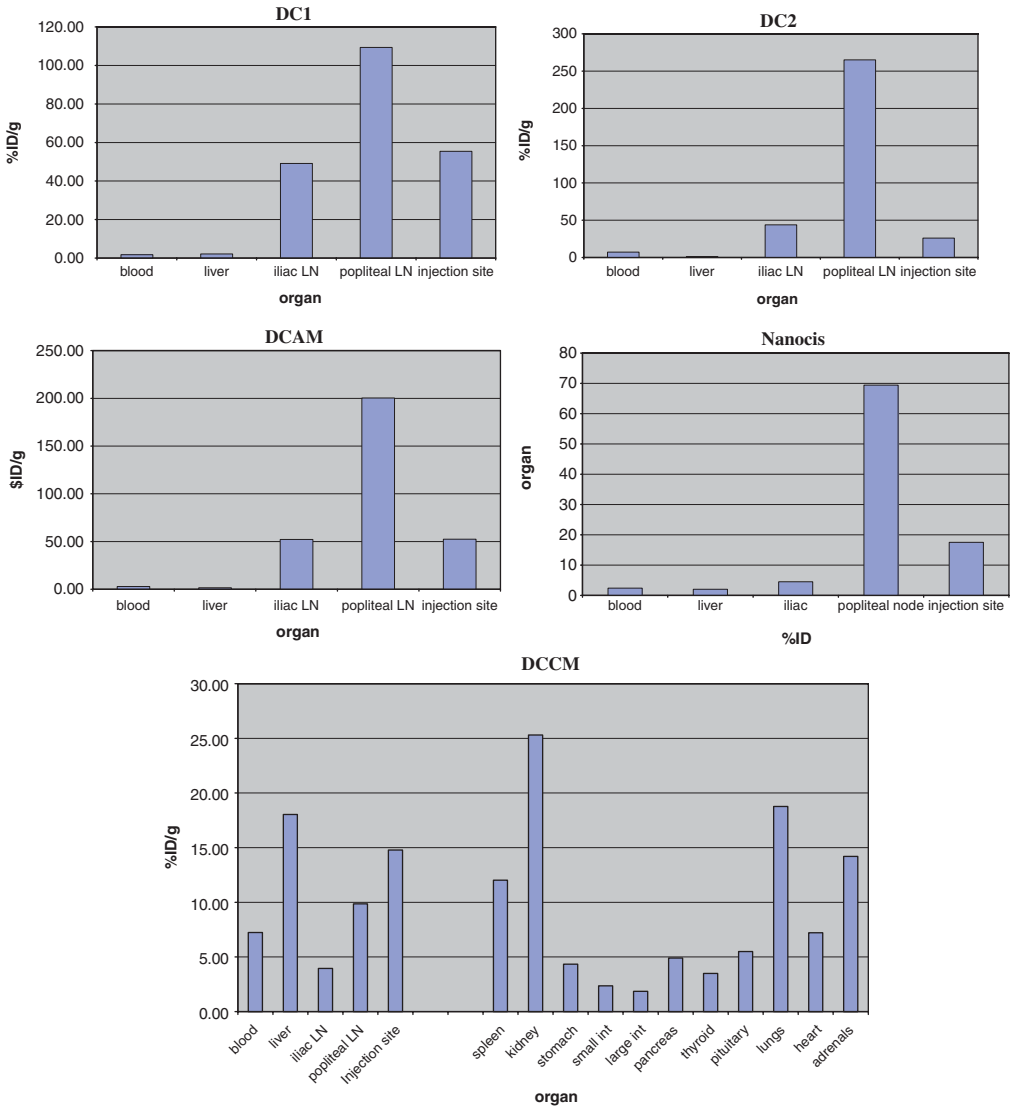

Figure 4. Biodistribution of DC1, DC2, DCAM, Nanocis and DCCM.

Table 4. Popliteal extraction

Probe	% ID SLN	% ID 2LN	E1 (%)
DC1	7.52	1.23	83.64
DC2	4.52	1.13	75.00
DCAM	3.89	1.57	59.83
DCCM	0.82	0.72	12.95

For comparative evaluation, the biodistribution of Nanocis in the same organs are presented. The results show very good uptake of the radiocolloid in the first lymph node (popliteal node), up to 5,9% ID (78.9% ID/g organ) while only 0.05% ID (2.2% ID/g organ) was found in the second lymph node in the chain (inguinal node).

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MAKING THE TRANSITION FROM PREPARING RADIOCHEMICALS WITH SOFT BETA TO THOSE WITH HIGH-ENERGY EMITTING ISOTOPES

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Summary : Successfully adjusting to the title transition requires not only making several general procedural changes but significant modifications in attitude. These procedural changes include major modifications in radiation safety practices. Modeling these reactions using tritium is often useful for both developing the radiochemistry and in making products useful in studies to determine if a given compound is likely to work as a PET or SPECT ligand. The required major change in attitude stems from the impact of having a significant external radiation hazard. There are also a number of ways in which working with these are similar or identical. Much of the knowledge and skill gained in working with soft beta emitters is directly applicable to work with high energy isotopes especially with regard to radiation safety issues. Practices like frequent glove changes, checking for the possible spread of contamination during the work day and carefully surveying the work area at the end of the day apply equally well to both. Examples of syntheses of this type along with the necessary practical considerations will be given.

Key Words: Radiation exposure/hazard; tritium; PET/SPECT; soft beta emitters; high energy isotopes; syntheses

Introduction: Major consideration must be given to the ways that the radiation safety and technical issues are different or similar for syntheses with these two types of isotopes. Concerns about these issues were initially addressed by visits to the PET/SPECT synthesis labs at the University of Wisconsin Medical Center, Cellectar and Johns Hopkins University School of Medicine where syntheses of [O-^{11}C-methyl]raclopride, [^{18}F]azan and [^{68}Ga]DOTA-antibodies were observed. The knowledge gained from these was then applied to the laboratory set up and execution of syntheses at Abbott of [^{123}I]-5-1A and ^{111}In-DTPA-antibodies.

Results and Discussion: The principal radiation safety differences include the need for lead shielding and adapting to working in what is often a confined area with limited visibility. Procedures must be designed and carried out where minimizing external exposure is considered, e. g., extensive use of tongs to handle reaction vessels. Manipulations must be minimized as much as possible by rehearsing and doing many trial runs where movements need to be carefully planned and executed.

There are also significant similarities. The basic aspects of radiation safety certainly apply, e. g., a healthy respect for the radiation hazard involved, frequent glove changes and surveying for possible contamination (easier with higher energy isotopes) as well as a thorough survey of the work area at the end of the work day.

Modeling synthetic procedures with high specific activity tritium often provides products that are used in studies which indicate whether a particular compound might be good as a PET ligand. This is also useful in working out conditions that can be applied to PET labeling, e. g., preparing high-specific activity [^{3}H]N-methylated compounds as analogs of their [^{11}C]N-methylated equivalents as PET ligands.

The recommended approach to undertaking this transition was followed which was observing established laboratories in action. The first visit was to the University of Wisconsin Medical Center and Cellectar in Madison, WI. Good ideas were obtained about the design and equipping of the laboratory, what the lead shielding requirements are, and design as well as use of a simple radio-HPLC detector and radio-TLC scanner. Also learned were some things to be avoided, a rough idea of how much activity can be handled safely without a hot cell and what the practical aspects of radiolabeling of this type are. Work in a high level hot cell was observed, as well. The next visit was to Johns Hopkins University School of Medicine in Baltimore, MD where actual [^{11}C]PET labeling runs were observed. The very close team work needed to get these syntheses and purifications done fast enough was impressive to watch. The emphasis was on the need to work "efficiently" rather than quickly. It was useful to see what it was like to work in a different type of "hot cell" than the one at Celectar. A laboratory where ^{68}Ga labeling of antibodies was being done using a generator was very interesting to see. Actual ^{18}F syntheses were also observed and a laboratory for studying the metabolism of PET ligands (mostly devoted to determining how much of the ligand gets into the brain in animal studies) visited.

The first run observed at Johns Hopkins was the preparation of [O-^{11}C-methyl]raclopride. The synthesis has to be extremely efficient and fast due to the short $t_{1/2}$ of ^{11}C ($\sim$20 min). It was done in 30 min from [^{11}C]CO_2 production from the cyclotron to dosing the patient. Effective teamwork was critical to getting this done with several tasks being done simultaneously. Minimizing exposure to external radiation required quick and error free handling. Quality control was very important to insure quality of the product and safety of the patient, e. g., radio-HPLC and GC analyses as well as pyrogen testing. Next a synthesis of [^{18}F]azan[1] was observed which was done in about one hour using a computer-controlled apparatus specifically designed for this due to it's 511 keV β^{+} radiation. Several leaving groups had been tried in model runs and -$^{+}$N(Me)$_3$ was found to be the best. Good specific activity was achieved. The HPLC system had to be very compact to fit into the hood and was linked directly to the apparatus. This featured a very simple injector and small UV detector. The product was collected from the prep HPLC purification using a switching valve on the apparatus. It was pointed out that it's important to be very careful to avoid lowering specific activity of the product due to trace amounts of F^{-} in water, plastic tubing, etc. Many practice runs had to be done to make it possible to get it done fast enough due to the short ($\sim$110 min) $t_{1/2}$ of ^{18}F. Radiolytic decomposition of the product is much more severe in this and all PET/SPECT labeling than with softer isotopes like ^{3}H and ^{14}C and $>$95% radiochemical purity is usually considered acceptable. Even for routinely prepared compounds such as [^{18}F]FDG usually a radiochemical purity of $>$95% or even less sometimes (minimized by diluting product solution as much as possible) is considered acceptable.

The first preparation done at Abbott was that of the well known SPECT ligand, [^{123}I]-5-1A[2]. There were other radiation safety issues in this work due to the volatile activity observed. A special charcoal hood filter capable of trapping volatile ^{123}I was required to keep it from getting into the environment. Thyroid monitoring was necessary to check for possible exposure. A preparative HPLC purification was required. However, the lead shielding requirement was not too large for the 159 keVν radiation and a hot cell or special apparatus was not required.

Subsequently, several ^{111}In-DTPA modified antibodies were prepared for small animal SPECT studies. The reasons for choosing ^{111}In for antibody labeling include the characteristics of the isotope (decay of $\sim$171 and 246 keVν which makes it suitable for imaging), $t_{1/2}$ = 2.80 d, stability of complexes with chelating agents like DTPA at room temperature in aqueous solution near neutral pH (see Figure 1), no problem with dilution due to unlabeled In in the environment and the fact that it is cheap as well as readily available[3]. The results are highly dependent on the quality of the DTPA-modified antibody. The ideal modification is $\sim$5-7 DTPA residues/antibody (usually doesn't perturb the immunoreactivity enough to be a problem). The aqueous medium is also very important since it must not complex or chelate the ^{111}In. We have found 0.1*M* NaOAc to be optimal. The best conditions for the reaction were found to be room temperature with thorough mixing for $\sim$10 min. The ideal final pH is $\sim$5-6. Typically $>$95% ^{111}In-DTPA antibody+aggregate is formed under these conditions but sometimes not (see Figure 2). A specific activity $>$20 μCi/μg is required for dosing. The very delicate nature of ^{111}In-DTPA antibodies must be considered in choosing a purification method. Hence, very gentle and rapid methods must be used that give a final concentration $>$2.5 mCi/mL for dosing animals. For this reason preparative SEC HPLC which results in a larger volume of product solution is not very useful whereas an SEC cartridge column followed by centrifugation works very well (see Figure 3). Storage for several hours at room temperature leads to significant changes in composition (see Figure 4).

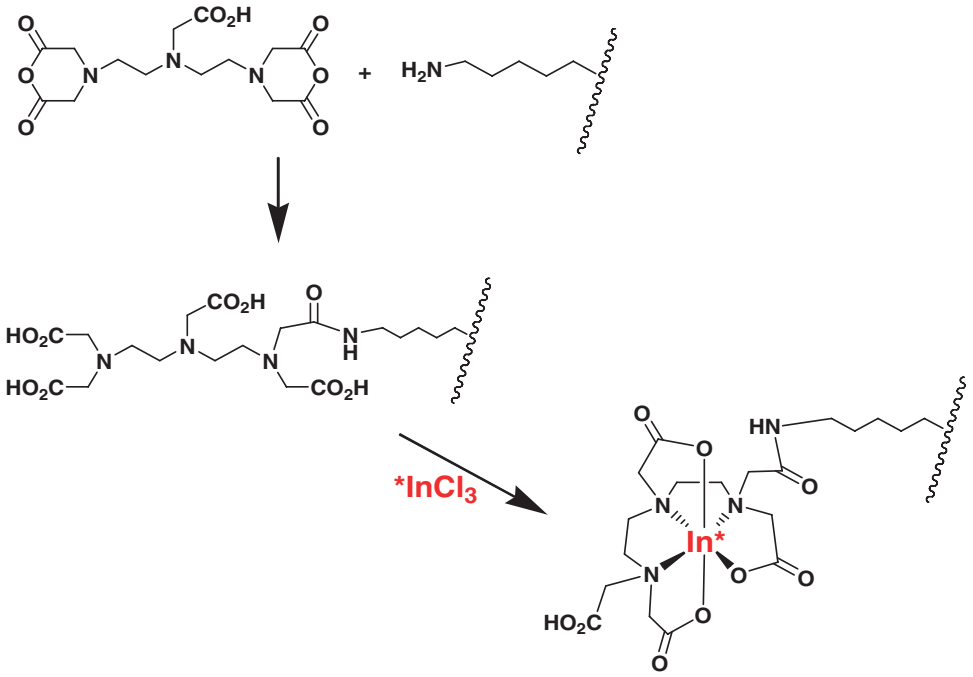

Figure 1. Synthesis of ^{111}In-DTPA(Diethylenetriaminopentaacetic acid) Antibodies.

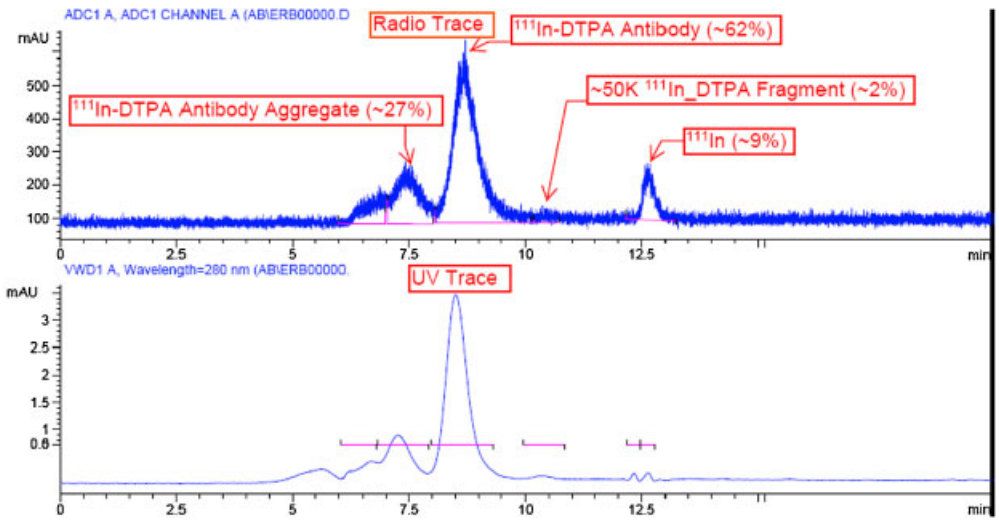

Figure 2. SEC RHPLC of an ^{111}In-DTPA-Antibody- Crude Reaction Mixture.

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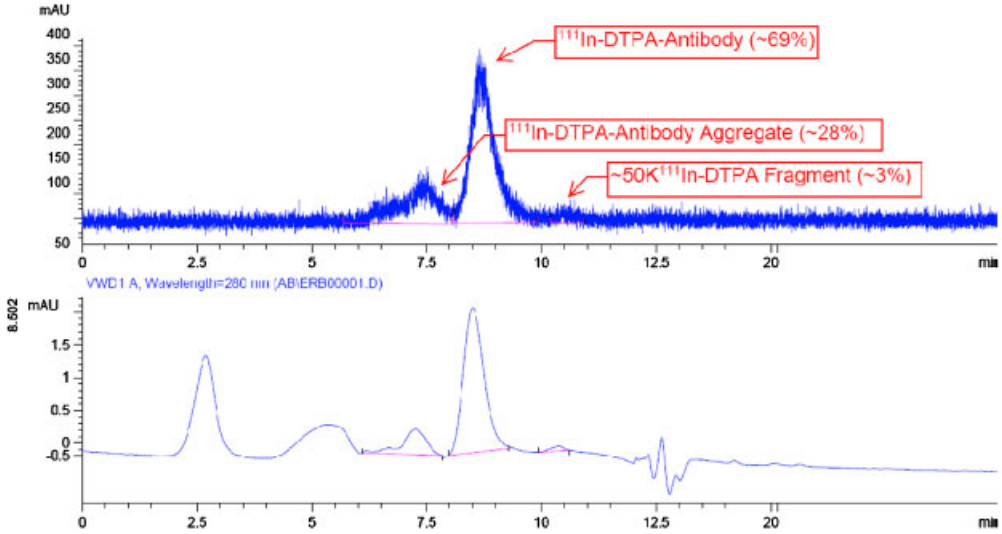

Figure 3. SEC RHPLC of Purified ^{111}In-DTPA-Antibody.

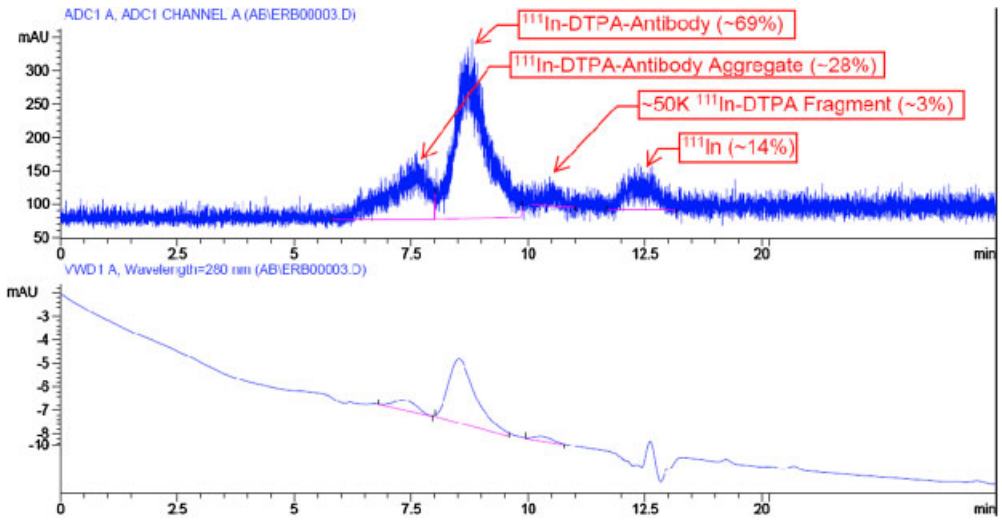

Figure 4. Purified 111In-DTPA-Antibody after storage for 42 hr at Room Temperature.

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