IAEA-SR-209/37

Labeling of MoAb with ^{153}Sm-HETA

G. FERRO-FLORES[1], F. De M. RAMIREZ[1], M. PEDRAZA-LOPEZ[1], J. I. TENDILLA[1], L. MELENDEZ-ALAFORT[2], C.A. MURPHY[2]

[1]National Institute of Nuclear Research (ININ), Edo. de Mexico, MEXICO

[2]National Institute of Nutrition "Salvador Zubirán" (INNSZ), Mexico City, MEXICO

A method to label MoAb with Sm-153 using 1, 5, 9, 13-tetraazacyclohexadecane N,N',N",N"' tetraacetic acid (HETA) as a new bifunctional chelator was developed. HETA was synthesized in our laboratory by reaction between chloroacetic acid and ano-N4 ligand in aqueous solution at 0°C overnight followed by a precipitation at pH 2.0 and dried under vacuum (m.p. 242-244°C). The product was characterized by IR, RMN and thermogravimetric analyses showing high purity [1].

Samarium-153 chloride was obtained by neutron irradiation of 10 mg of enriched Sm_2O_3 (^{152}Sm, 99.4 %, from ISOTEC Inc.) in a Triga Mark III reactor at a flux in the central thimble of $3x10^{13}$ n cm^{-2} s^{-1} for 20 h. [2] After irradiation 100 μL of 12 N chloride acid was added to the irradiation vial and stirred for 1 min followed by the addition of 900 μL of injectable water and also stirred for 2 min. The average radioactive concentration was 37 GBq/mL.

Sterile and apyrogenic V vials were prepared to contain 1.0 mg ($2.17x10^{-3}$ mmoL) of HETA in 1.0 mL of 0.5 M bicarbonate buffer (pH 8.3) plus 20 μL of 2.5 N NaOH then 10 μL of $SmCl_3$ solution ($4.9x10^{-4}$ mmoL Sm, 370 MBq) was added and the mixture, with a final pH 9.0, was incubated at 78°C for 3 h. Radiochemical purity was evaluated by TLC utilizing aluminum cellulose sheets (Merck) as the stationary phase with methanol : water : ammonium hydroxide (20 : 40 : 2) as the mobile phase. Sm^{+3} remained at the origin (R_f = 0) and ^{153}Sm-HETA traveled with the solvent front with a R_f value of 0.9-1.0.

Murine monoclonal antibody (MoAb) IgG1 ior ceal against carcinoembryonic antigen (CEA) was supplied by the Center of Molecular Investigations (CIMAB, Havana, Cuba) into vials containing 5.0 mL of a sterile and apyrogenic neutral phosphate buffer saline (PBS) solution with an antibody concentration of 1.0 mg/mL. To 1.0 mL of MoAb solution was added 1.0 mL of ^{153}Sm-HETA solution and the mixture was incubated at room temperature (18-20°C) until 24 h. Quality control of the labeled antibody was evaluated by size exclusion HPLC analysis employing a ProteinPak 125 SW gel filtration column (Waters), with photodiode array detector. 0.1 M phosphate pH 7.4 at a flow rate 1.5 mL/min was used as mobile phase. Under these conditions Sm^{+3} was retained into the column and for MoAb and ^{153}Sm-HETA the retention time was 4.5 min and 6.9 min respectively (Fig. 1). The radiochromatographic profile was determined by collecting samples (Waters fraction collector) of uniform volume (0.5 mL) for counting in a external NaI (Tl) detector (NML, Laboratories, Inc.).

Radiochromatographic profile showed that 10 min after incubation only 15.6% of the radioactivity was associated with the MoAb (Fig. 2A) and after 24 h it increased to 95% (Fig 2B). Under these conditions 0.628 mol and 3.5 mol of ^{153}Sm-HETA were coupled to each mol of MoAb after 10 min and 24 h respectively. The formation of ^{153}Sm-HETA labeled MoAb by a simple incubation of the antibody with the samarium complex, even when ^{153}Sm-HETA was prepared as a stable complex, could be explained on the basis that one HETA carboxyl group does not participate in neutralizing the charge of the metal and it is available to react with the amino groups of the antibody.

The specific activity of the labeled antibody was 111 MBq/mg (3 mCi/mg). Sm-153(III) is commercially available with specific activities up to 318.2 GBq/mg (Oak Ridge National

Laboratory). Therefore, under the conditions described above ^{153}Sm-HETA labeled MoAb could be obtained with specific activity up to 1.14 GBq/mg (30.7 mCi/mg).

In order to establish the therapeutic possibilities for ^{153}Sm-HETA labeled MoAb obtained in this study it will be necessary to perform studies in normal and tumor-bearing mice.

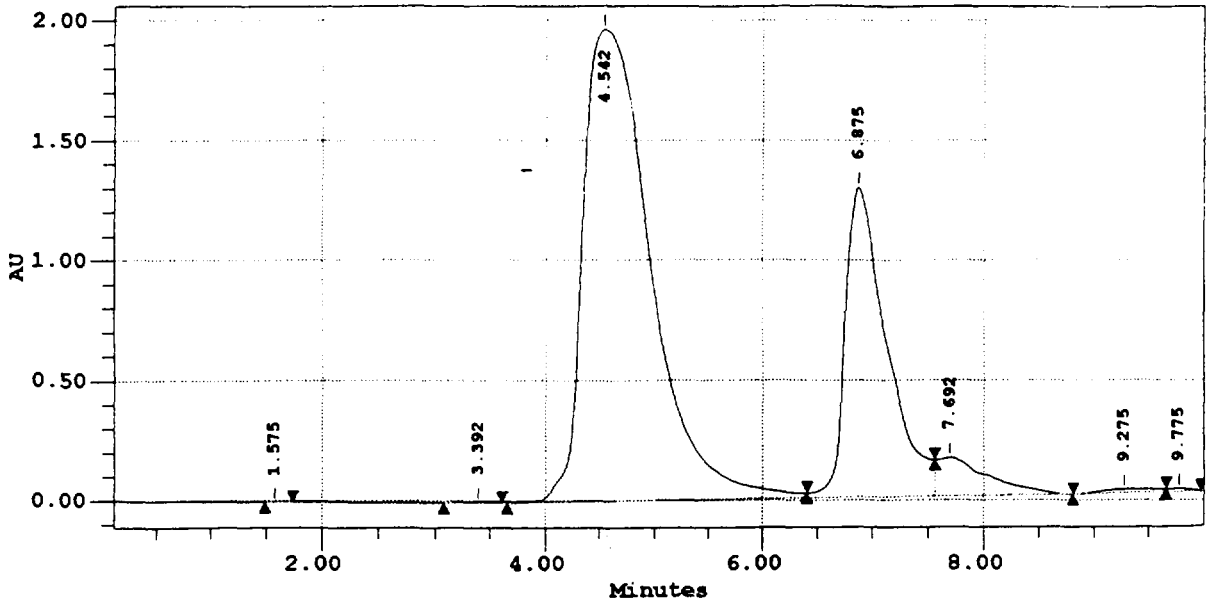

Fig. 1. HPLC separation (UV detector) of MoAb (Tr=4.5 min) and ^{153}Sm-HETA (Tr=6.9 min)

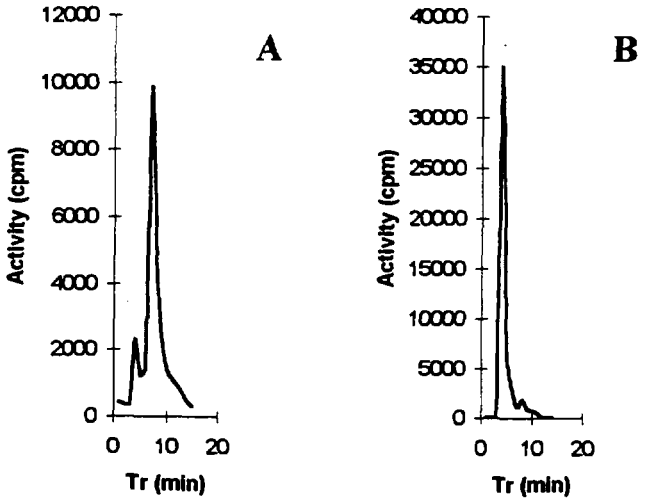

Fig. 2. Radiochromatographic profile obtained during the preparation of ^{153}Sm-HETA labeled MoAb A) 10 min after incubation B) 24 h after incubation.

References

1. F. de M. Ramirez. The reactivity of the lanthanide metal ions towards tetraazamacrocyclic ligands. Mexican National University, 1996 (PhD Thesis, in Spanish).
2. G. Ferro-Flores, J.I. Tendilla, M.A. López-Gómez, M.A. González-Zavala, L. Paredes Gutiérrez, E. Avila-Ramírez and F. Aguilar-Hernández. Kit Preparation of ^{153}Sm-EDTMP and Factors Affecting Radiochemical Purity and Stability. J Radioanat *Nucl Chem Articles* 1996;204(2):303-311.