LABELLING OF MoAb WITH $^{153}SmH_1ETA$: PRELIMINARY RESULTS

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Abstract. A method to label MoAb with Sm-153 using 1,5,9,13-tetraazacyclohexadecane N,N',N'',N''' tetraacetic acid (H_4ETA) as a bifunctional chelator was developed. H_4ETA and SmH_1ETA were synthesized in our laboratory and characterized by IR spectroscopy, TGA (thermogravimetric analysis), SEM (Scattering Electronic Microscopy), EDAX (Elemental Dispersion Analysis by X-rays) and EPR (Electron Paramagnetic Resonance) at 6 K. The $^{153}SmH_1ETA$MoAb was prepared by a simple incubation of the MoAb ior cea1, and the $^{153}SmH_1ETA$ complex at neutral pH and at room temperature for 24 h. The specific activity of the ^{labelled} antibody was 111 MBq/mg (3 mCi/mg). Sm-153(III) is commercially available with specific activities up to 318.2 GBq/mg. Therefore, under the conditions described above $^{153}SmH_1ETA$ ^{labelled} MoAb could be obtained with specific activity up to 1.14 GBq/mg (30.7 mCi/mg).

1. INTRODUCTION

The chelators used successfully to radio^{labelled} biologically important molecules such as antibodies and peptides with ^{99m}Tc are amine oximes (HMPAO), diaminothiols (DADT, BAT), hydrazinonicotinamide (SHNH, hynic), thiosemicarbazones as well as amidothiols like mercaptoacetyltriglicine (MAG_3)[1]. However when the biomolecules are required for therapeutic purposes, the traditional chelators are not the most appropriate. For example, despite the similarities in the chemical properties of rhenium and technetium, the labelling procedure employing Re has to be carried out by a multistep procedure and under acidic conditions which could affect the biomolecule integrity [2].

Whereas Samarium-153 has several favorable features as a radiotherapeutic agent. It possesses a 103 KeV gamma emission for scintigraphic imaging of its biological distribution allowing the *in vivo* absorbed dose calculation. The short physical half-life ($t_{1/2}$ = 1.8 days) of ^{153}Sm reduces the need for long patient isolation and facilitates the disposal of urine and other body fluid. ^{153}Sm gamma emission makes it to be a radionuclide of easy handling from the point of view of radiation protection. Due to its cross section (σ = 260), a reasonable high specific activity of samarium-153 can be produced even with fairly low flux nuclear reactors, which allow practical applications in nuclear medicine.

The aim of this work was to synthesize H_4ETA as a bifunctional chelator in order to examine the feasibility of labelling monoclonal antibodies (MoAb) with Samarium-153 under safety reaction conditions such as room temperature and neutral pH. For this purpose the synthesis and characterization of SmH_1ETA complex in macroscopic quantities were performed.

2. MATERIALS AND METHODS

2.1 Synthesis of H_4ETA.

H_4ETA was synthesized in our laboratory by reaction between chloroacetic acid and ano-N_4 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) [3]. The product was characterized by IR, RMN and thermogravimetric analyses.

2.2 Synthesis and characterization of SmH_1ETA.

The SmH_1ETA complex was prepared according to the reported in the literature [4]. A schematic representation of the route synthesis for the SmH_1ETA complex is as follows:

$SmCl_3 \bullet \times H_2O/H_2O + H_4ETA/NaOH/pH = 10 \rightarrow$ Solution/pH = 6–6.7 $\rightarrow$ Sol. Mixture

Sol. Mixture reaction/80–85^{0}C, 17 h $\rightarrow$ concentration/vacuum at 45^{0}C $\rightarrow$ SmH_1ETA

2.3 Preparation of $^{153}SmCl_3$ solution.

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 3×10^{13} n· cm^{-2}· s^{-1} for 20 h. [5]. 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.

2.4 Preparation of $^{153}SmH_1ETA$ complex.

Sterile and apyrogenic V vials were prepared to contain 1.0 mg (2.17×10^{-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 (4.9×10^{-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 $^{153}SmH_1ETA$ traveled with the solvent front with a R_f value of 0.9–1.0.

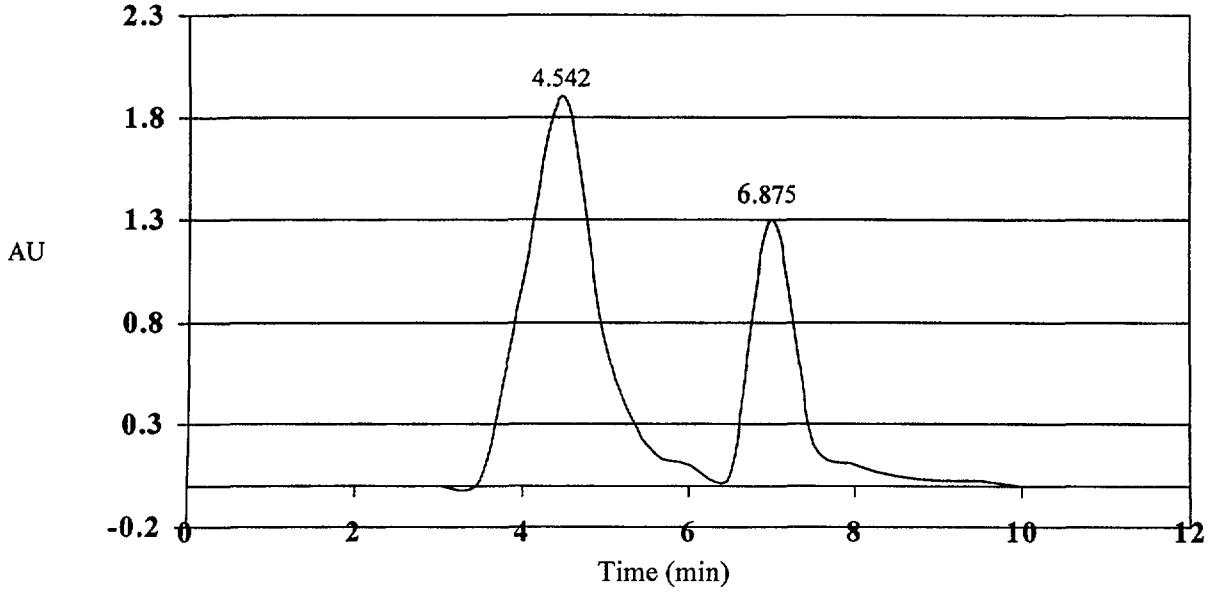

FIG. 1. HPLC separation (UV detector) of MoAb (Tr=4.5 min) and ^{153}Sm-HETA (Tr = 6.9 min).

2.5 Preparation of $^{153}SmH_1ETAMoAb$.

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 $^{153}SmH_1ETA$ solution and the mixture was incubated at room temperature (18–20°C) and neutral pH until 24 h.

2.6 Radiochemical quality control

Quality control of the ^{labelled} 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 $^{153}SmH_1ETA$ 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.).

3. RESULTS AND DISCUSSION

The H_4ETA and the SmH_1ETA complex were obtained as reported elsewhere [3] and [4], respectively. Their preliminary characterization were carried out by IR spectroscopy, TGA (thermogravimetric analysis), SEM(Scattering Electronic Microscopy), EDAX (Elemental Dispersion Analysis by X-rays) and EPR(Electron Paramagnetic Resonance) at 6 K. Their IR spectra are shown in FIG. 2a,b ; as it is seen the formation of the complex modified the spectrum of the ligand. The main vibration frequencies assigned to O-H, CH_2-C=O, $O=CO^{-1}$ in the free ligand (3456, 2969 and 1647 cm^{-1} respectively) were shifted to lower energies and those corresponding to C-N-C (1126, 1036 cm^{-1}) and -CH_2-CH_2-(916, 704 cm^{-1}) to higher energies. As it was observed in other similar LnHETA complexes [4] the CH_2-N- band at 1477 cm^{-1} disappeared. This indicated the geometry change of the ligand after Sm(III) was coordinated. It is worthwhile to mention that a band between 2480–2290 cm^{-1} (corresponding to NH^+ group) did not disappear completely after coordination which suggested the presence of HOOC group, this is revealed by the formation of a zwitterion (NH^+-OOC) in the KBr matrix. No free ligand and nor Sm-Cl vibration frequencies corresponding to $SmCl_3$ were observed in the complex.

Semiquantitative microelemental analysis by EDAX, the minimum formula reported for similar compounds [4] and the TGA let us to propose that the complex was stabilized as $SmH_1ETA \cdot 3NaCl \cdot 3H_2O$. The feature of the EPR spectrum of this complex (FIG. 3) also suggested that samarium(III) was coordinated to the ligand. Besides, the SEM picture (FIG. 4) showed a homogeneous topology of the sample, which evidences an acceptable purity of one unique complex.

So far we can propose that the SmH_1ETA complex can be conjugated to the MoAb antibody through its no ionized HOOC- group, and thus allowed the labelling of the associated species as $^{153}SmH_1ETAMoAb$.

During the radiolabelling procedure, radiochromatographic profile showed that 10 min after incubation only 15.6 $\pm$ 3.2 % of the radioactivity was associated with the MoAb (FIG. 5A) and after 24 h it increased to 95 $\pm$ 2.1 % (FIG. 5B). Under these conditions approximately 0.628 mol and 3.5 mol of $^{153}SmH_1ETA$ were coupled to each mol of MoAb after 10 min and 24 h respectively. The formation of $^{153}SmH_1ETA$ ^{labelled} MoAb by a simple incubation of the antibody with the samarium complex, even when $^{153}SmH_1ETA$ was prepared as a stable complex, could be explained on the basis above described.

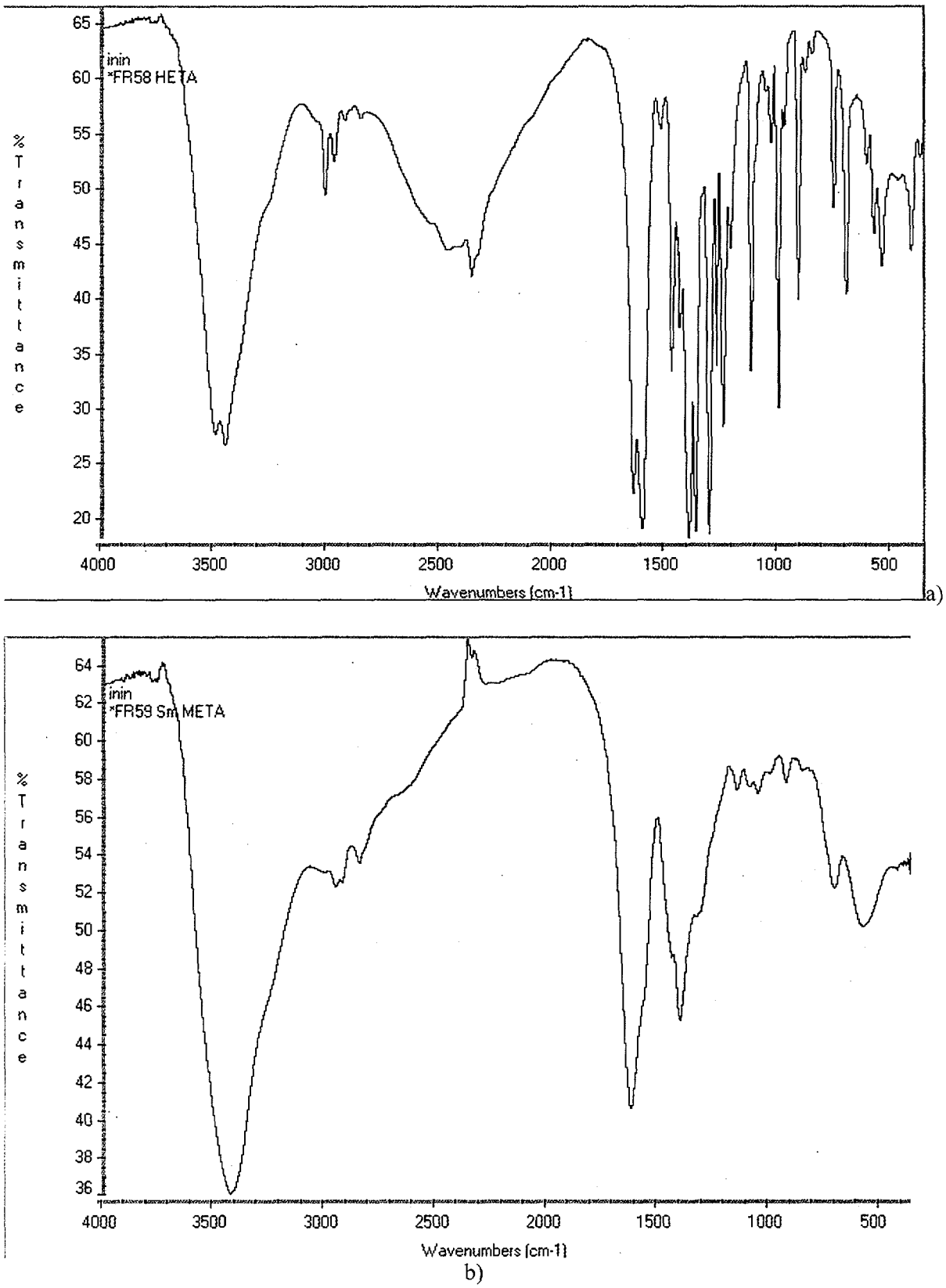

FIG. 2. IR spectra in KBr matrix of a) H_4ETA and b) $SmH_1ETA \cdot 3NaCl \cdot 3H_2O$.

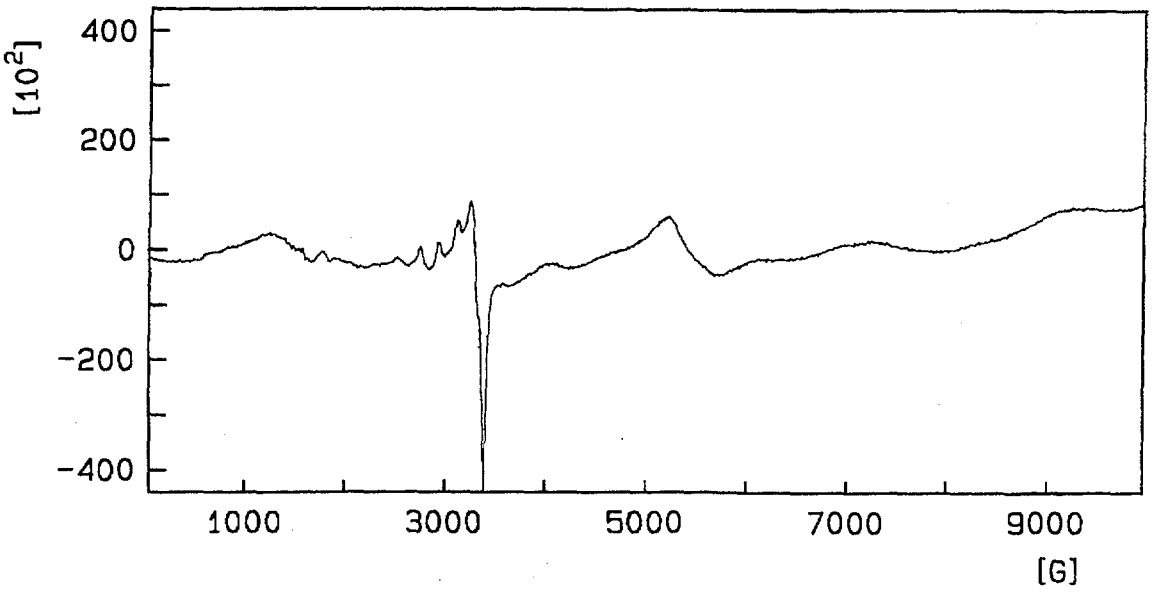

FIG. 3. EPR spectrum at 6 K of $SmH_1ETA \cdot 3NaCl \cdot 3H_2O$.

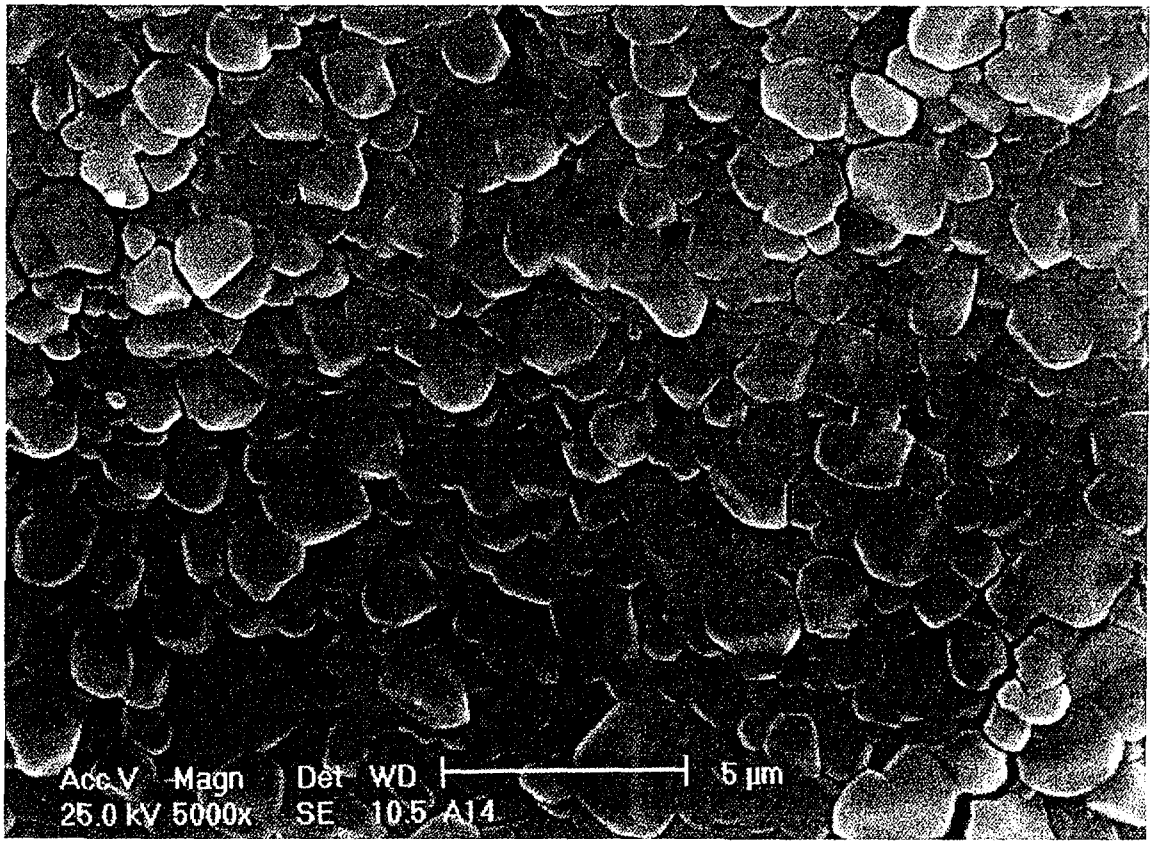

FIG. 4. SEM picture of $SmH_1ETA \cdot 3NaCl \cdot 3H_2O$.

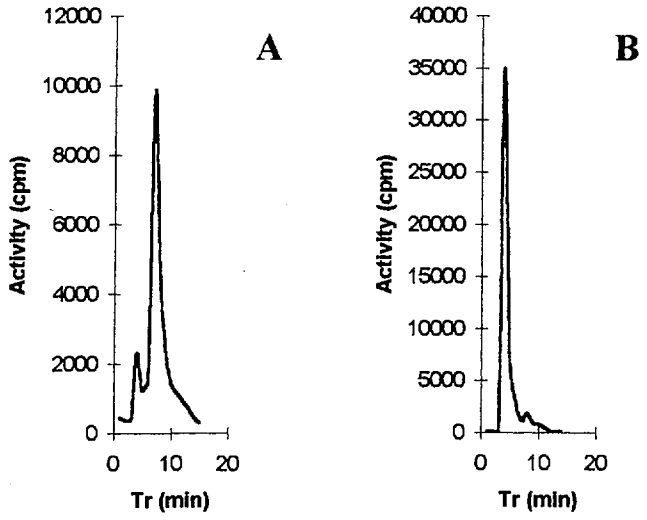

FIG. 5. Radiochromatographic profile obtained during the preparation of $^{153}SmH_1ETA$ ^{labelled} MoAb A) 10 min after incubation B) 24 h after incubation.

The specific activity of the ^{labelled} 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}SmHETA ^{labelled} 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 $^{153}SmH_1ETA$ ^{labelled} MoAb obtained in this study it will be necessary to perform studies in normal and tumour-bearing mice.

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