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# METHOD FOR EFFECTIVE $^{201}$Tl(III) LABELLING OF DIETHYLENETRIAMINE PENTAACETIC ACID (DTPA)-FUNCTIONALIZED PEPTIDES: RADIOSYNTHESIS OF $^{201}$Tl(III)DTPA-NEUROTENSIN(8-13).

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## SUMMARY

This paper describes a unique approach to the $^{201}$Tl(III) complexation of peptides, starting from the commercially available thallous(I)chloride ($^{201}$Tl). The latter was first of all oxidised using $O_3$ and the thallium(III) salt complexed with diethylenetriamine pentaacetic acid neurotensin(8-13) (DTPA-NT(8-13). Radiolabeling yields of 97.5% could be obtained within 5 minutes and the complex was stable for at least 48 hours. The strategy used is potentially applicable to many new peptide derived radiopharmaceuticals.

*Keywords:* $^{201}$Tl(III) complexation, DTPA, Neurotensin(8-13).

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## INTRODUCTION

Thallium-201 ($^{201}$Tl) can be used as a specific radioisotopic tracer both for heart and tumour scintigraphy. Due to its increasing availability, $^{201}$Tl might become an interesting alternative for labelling functionalized molecules with Indium-111 ($^{111}$In). $^{201}$Tl has interesting gamma-energies and is an X-ray emitter with a half-life of 3.04 days which makes it useful for diagnostic nuclear medicine including tumour localisation in humans.

Many tumours have been characterised by an over expression of neuropeptide receptors which makes neuropeptides interesting molecules in providing new approaches for radiopharmaceutical development (1-4).

Neurotensin (NT) is a thirteen amino acid (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) neuroendocrine peptide which has been shown to be involved in the development and the metastasis of different human tumours including Ewing's sarcoma, non-endocrine pancreas tumours and meningioma (5). As a result, radiolabelled neurotensin or neurotensin derivatives might be potentially useful vectors for localisation and internal radiotherapy of the above mentioned neurotensin receptor expressing pathologies. For many years NT has been the neuropeptide of our interest: different approaches have led to the synthesis and radiosynthesis of radiopharmaceuticals derived from the biological active part of NT, H-Arg$^{8}$-Arg$^{9}$-Pro$^{10}$-Tyr$^{11}$-Ile$^{12}$-Leu$^{13}$-OH or NT(8-13) (6-8). Among different functionalized NT(8-13) analogues, diethylenetriamine pentaacetic acid neurotensin(8-13) (DTPA-NT(8-13) was introduced as a target specific functionalized neurotensin derivative for radiolabelling with trivalent metal radioisotopes. The combination of the availability of a target specific tracer on the one hand and an interesting radioisotope on the other hand led us to investigate the possibility of complexing DTPA-NT(8-13) with $^{201}$Tl(III). We report here on a strategy for a successful $^{201}$Tl(III)-complexation starting with the commercially available $^{201}$Tl(I)chloride. We propose a unique radiolabelling approach which potentially can be applied to many polyamino polycarboxylate complexed

 

peptides and which offers an alternative for labelling with other metal radionuclides including [$^{111}$In].

## EXPERIMENTAL

*Peptide Synthesis*

DTPA-NT(8-13) was prepared by solid phase synthesis on a Merrifield resin using Boc-main chain protection as described in detail elsewhere (5,7). After synthesis, DTPA-NT(8-13) was purified by HPLC using a 5μ Vydac reversed phase C-18 column. Quality control was performed on the lyophilised purified peptide using electron spray LC - MS.

*Thallium(III) production*

$^{201}$Tl(I)chloride (DRN 8103 Thallous chloride injection) was provided by Mallinckrodt (Petten, The Netherlands). To 75MBq of $^{201}$Tl(I)chloride (37MBq in 1ml 0.9% NaCl), 2ml of 0.2N HCl was added. This mixture was treated with ozone ($O_3$) for 1 minute. $O_3$ was produced from medicinal oxygen (Air Liquide, Belgium) using a conventional $O_3$ generator at a flow rate of 1liter per minute. During $^{201}$Tl(I) oxidation, any contact with metal containing materials was avoided. After ozonification, the obtained $^{201}$Tl(III)chloride solution was treated directly with $N_2$ in order to remove any traces of $O_3$ in the thallium-HCl-solution. $N_2$ was passed through the $^{201}$Tl(III)chloride solution at a flow rate of 1liter per minute for 1minute. Again, contact with metal containing materials was avoided. The quality of the oxidation of $^{201}$Tl(I) was checked by cellulose acetate paper electrophoresis (Gellmann) in 0.05N EDTA at 200V for 10 minutes. The produced Tl(III) solution was checked for any traces of $O_3$ before further use. Therefore, 1μl samples of the freshly produced $^{201}$Tl(III) solution were added to 25μl of a 5M NaI-solution: traces of $O_3$ turn the NaI-solution a dark yellow colour.

*Thallium (III) complexation of DTPA-NT(8-13): KIT preparation*

To a kit containing 20μg (0.8nmol) of DTPA-NT(8-13) and 5mg of sodium citrate in 1ml of 0.9% NaCl, 37MBq of [$^{201}$Tl](III) in 0.1N HCl were added. The pH of the reaction mixture was 4.5. After

 

homogenisation, the reaction mixture was left at room temperature for 5minutes. 5µl Aliquots of the reaction mixture were HPLC analysed using a 5µ Vydac RP C-18 column (4.6mm by 250mm) in $H_2O$/ACN//TMA/AcOH 80/20//1.5/2 (v/v//v/v) of pH 4.2 (flow rate = 1ml/minute).

*Shelf-life stability of $^{201}$Tl(III) chloride and $^{201}$Tl(III)-DTPA-NT(8-13)*

The shelf-life stability of $^{201}$Tl(III) chloride was studied with the proposed cellulose acetate electrophoresis method and that of the $^{201}$Tl(III)-complexed DTPA-NT(8-13) by analytical HPLC. $^{201}$Tl(III) was stored at room temperature as a 0.1N HCl solution whilst the $^{201}$Tl(III)-DTPA-NT(8-13) was stored at room temperature in its kit solution.

## RESULTS

Table 1 gives the analytical quality control data based on electrophoresis. In a 0.05N EDTA solution, $^{201}$Tl(III) forms $^{201}$Tl(III)EDTA-complexes which migrate on a cellulose acetate strip towards the anode. An average of 98% (min. 93.9%, max 99.9%) of Tl(III) is obtained using the proposed oxidation method. Fig 1 shows the analytical HPLC of $^{201}$Tl(III)DTPA-NT(8-13). At a $R_t$ of 2.4minutes, free $^{201}$Tl which most probably corresponds to traces of non-oxidised $^{201}$Tl(I) can be observed.

The amount of free $^{201}$Tl was always lower than 5%. DTPA-NT(8-13) and $^{201}$Tl(III)DTPA-NT(8-13) have a $R_t$ of 4.4minutes and 4.65minutes respectively. Average radiolabelling yields were around 97.5%. During radiolabelling, the reaction mixture remained colourless.

| Electrophoresis | Cathode | Anode | % free Tl(I) | % Tl(III) EDTA |
|---|---|---|---|---|
| cts1 | 15241 | 302565 | | |
| cts2 | 14525 | 302565 | | |
| cts3 | 15475 | 304256 | | |
| Average cts | 15080 | 303129 | 4.7% | 95.3% |

**Table1**: Quality Control Tl(III)chloride production based on cellulose acetate strip electrophoresis.

 

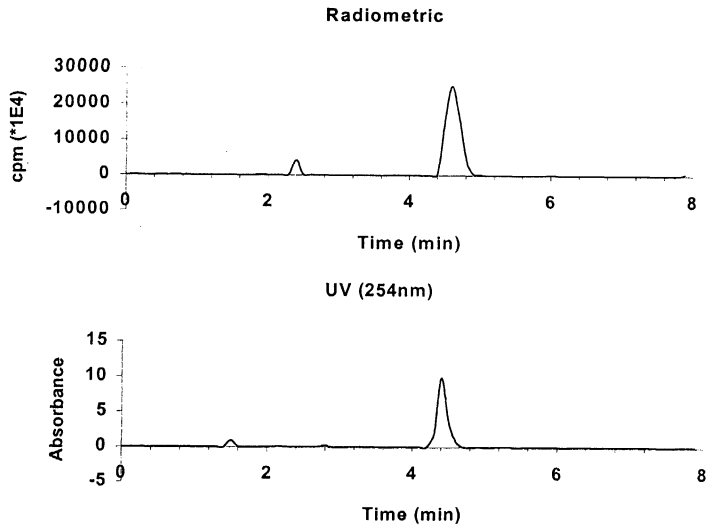


**Fig.1**: HPLC-analysis of $^{201}Tl$-DTPA-NT(8-13) on a Vydac RP-C18 column ($H_2O$/ACN//TMA/AcOH 80/20//1.5/2, pH = 4.2). UV at 254nM (upper) and radiometric (lower).

The shelf-life stability of the produced $^{201}Tl(III)$chloride is shown in Fig. 2. For at least 6 hours, $^{201}Tl(III)$chloride remains unchanged in its 0.1N HCl solution. For longer times, the quality of the $^{201}Tl(III)$ decreases mostly as a consequence of hydrolysis.

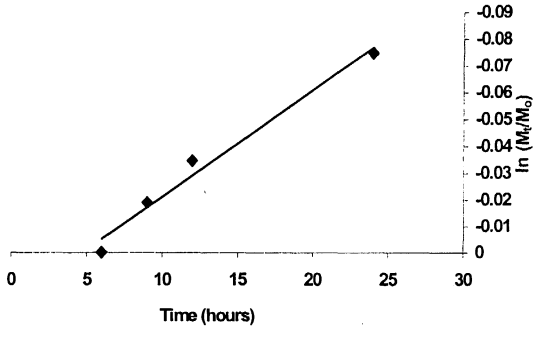


**Fig 2** : Shelf-life stability of Tl(III)chloride. Data based on electorphoretic analysis in 0.05M EDTA using cellulose acetate strips.

 

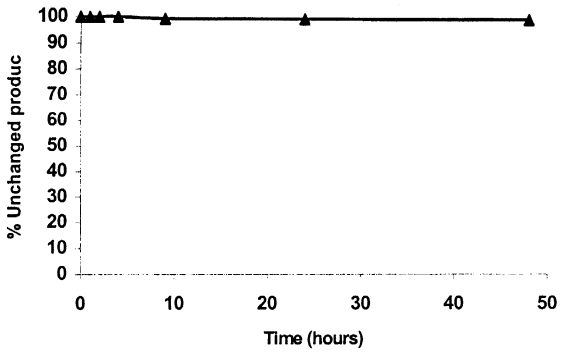


**Fig.3** Stability of $^{201}$Tl(III)-DTPA-NT(8-13) stored in a sodium citrate buffer at pH 5 at 4°C in a capped glass vial.

As seen from Fig 3, $^{201}$Tl(III)DTPA-NT(8-13) remains stable for at least 2 days when stored in a capped reaction vial containing a sodium citrate buffered solution at pH 5.

## CONCLUSIONS

This study shows that commercially available [$^{201}$Tl](I)chloride in 0.9% NaCl can be used to complex biomolecules containing a DTPA-bifunctional chelating system. $^{201}$Tl(I) oxidises in $^{201}$Tl(III) in no time and the labelling kinetics for $^{201}$Tl(III) complexation of DTPA-substituted molecules is very fast. Within 5 minutes, almost quantitative labelling of sub-nanomolar amounts of peptide can be achieved. Further *in vitro* and *in vivo* characterisation will be necessary in order to prove the potential usefulness of $^{201}$Tl(III)-DTPA-NT(8-13). Most probably, the DTPA-NT(8-13) analogue will need further modifications in order that it be clinically useful as the enzymatic stability of this compound is expected to be low. A combination of introducing both pseudo-peptide bonds and amino acid replacements could prove to be useful. The development of $^{201}$Tl(III)-complexed radiopharmaceuticals could provide many advantages: 1) the physical properties of $^{201}$Tl are interesting 2) the chemistry of $^{201}$Tl(III) complexation of DTPA-complexed molecules is simple 3) the complexation constant of $^{201}$Tl(III) for DTPA is among the highest (for

 

[ML] / [M] [L] at 25°C, log K = 46) for all metal-chelator complexes 4) due to the stability of the $^{201}Tl(III)$-DTPA complex, *in vivo* transchelation processes could be limited or avoided 5) high specific activity radiopharmaceuticals can be synthesised in kit formulations 6) [$^{201}Tl$] is available in many parts of the developed and developing world and can offer alternatives for other metallic radioisotopes including [$^{111}In$](III). Additionally, any new [$^{201}Tl$]-labelled compound can prove to be important in characterising pathologies and might be of use in any routine nuclear medicine department.

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