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# TRICARBONYLTECHNETIUM (I) LABELLED LIGANDS WITH NSO DONOR ATOM SET: *IN VITRO* AND *IN VIVO* EVALUATION

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## Abstract

There is an increasing interest for the $^{99m}$Tc labelling of biomolecules by using bifunctional chelating agents. To find new ligand, which can be linked to the small biomolecules and coordinated with technetium-99m tricarbonyl complexes, is a challenging task. The investigated NSC and NSC5 ligands allow the preparation $^{99m}$Tc(I) stable complexes in high yield. The $^{99m}$Tc complexes were characterized by comparing their HPLC profiles with those of the respective Re(I) compounds. Biodistribution and stability studies were carried out, including challenge with histidine. These complexes also proved to be stable in vivo and showed a very good biological behaviour. The radiochemical and biological features of the novel $^{99m}$Tc complexes, as well as, the nature of the ligands, make them very promising candidates for labelling of tumour specific biomolecules.

## Introduction

Technetium radiopharmaceuticals, as complexes of the $^{99m}$Tc radionuclide, are of great importance in diagnostic nuclear medicine. However, the use of $^{99m}$Tc for labelling small molecules, receptor ligands, has been rather limited. Over the last few years, the chemistry of a novel organometallic species, $M(CO)_3^+$ (M=Tc, Re), has been intensively developed. The water soluble technetium tricarbonyl complex $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was seen to be very versatile and effective precursor for labelling biomolecules [1]. The three coordinated molecules of water are labile and could be readily exchanged with various mono-, bi- and tridentate ligands. New chelating agents have been synthesized with the aim toward the design and development of site-specific radiopharmaceuticals [2], [3]. The aim of this study is to label ligands (2-benzimidazolylmethylthio) acetic acid (NSC) and N-1-Ethyl-(2-imidazolidinyl methylthio) acetic acid (NSC5) with $^{99m}$Tc (I)-precursor. The stability of the formed complexes and its in vitro and in vivo properties were investigated too.

## Materials and Methods

The samples of ligands (NSC, NSC5) were prepared by dissolving in water appropriate amount of substance for obtaining $10^{-3}$ mol dm$^{-3}$ solutions. pH was adjusted to 5.0. $^{99m}$Tc-carbonyl precursor was prepared according to manufacturer instruction (IsoLink$^{TM}$, Mallinckrodt Medical B.V., Netherlands). $^{99m}$Tc-(I) ligands complex were

prepared by addition of 0.1 ml of ligand solutions to 0.4 ml of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with appropriate pH values. The vials were heated for 30 min in boiling water bath. The labelling efficiency of $^{99m}$Tc-carbonyl targeted ligands was determined using gradient HLPC equipped with UV and radioactive γ-detector on Nucleosil 100-5 C-18 column. The 0.1% solution of TFA (trifluoroacetic acid) in $H_2O$ and 0.1% of TFA in acetonitrile were used as mobile phases.

TCA precipitation method for determining the percentage of $^{99m}$Tc(I)(NSC5) bound to proteins (12% human albumin, incubation at $37^0$C for different time intervals) was very useful [4]. All lipophilicity measurements were done by solvent extraction method with n-octanol equilibrated with 0.15 M phosphate buffers (pH=6.0-7.5. Organ biodistribution studies were carried out on white health Wistar rats (four weeks old). The animals were sacrificed 5 and 120 minutes after application of 0.1 ml of $^{99m}$Tc(I)(NSC5). The radioactivity per organ of interest was measured in a NaI (Tl) detector.

## Results

**Table 1.** Retention times and yields for $^{99m}$Tc complexes

| *Ligand (L)* | *$^{99m}$Tc species* | *$R_t$ (min)* | *Yield (%)* |
|---|---|---|---|
| | *$^{99m}TcO_4^-$* | - | - |
| *NSC* | *$[^{99m}Tc(CO)_3(H_2O)_3]^+$* | - | - |
| | *$^{99m}Tc(CO)_3$-NSC* | *13.74* | *100.0* |
| | *$^{99m}TcO_4^-$* | *4.00* | *3.2* |
| *NSC5* | *$[^{99m}Tc(CO)_3(H_2O)_3]^+$* | *13.12* | *2.9* |
| | *$^{99m}Tc(CO)_3$-NSC5* | *14.53* | *94.9* |

**Table 2.** UV chromatograms of Re(I) complexes

| *Re-complex* | *$R_t$(min)* | *Yield(%)* |
|---|---|---|
| *Re(I)-NSC* | *13.57* | *84.7* |
| *Re(I)-NSC5* | *14.39* | *95.1* |

The reactions produced a single product with yields greater than 90% (tab. 1, fig.1). The values of retention times varied for each complex. The identity of the $^{99m}$Tc complexes was established by comparative HPLC studies using samples of the well characterized rhenium (I) complexes as reference (tab. 2, fig.2). At 1, 6 and 24 hours post labeling the radiochemical purity of the formulations remained high and practically unchanged. Also, the complexes are resistant against histidine challenge.

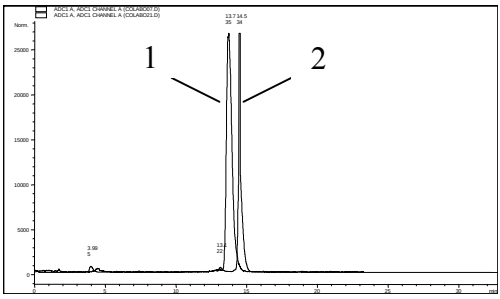


**Fig.1.** γ- chromatograms of $^{99m}Tc(CO)_3$-NSC (1) and $^{99m}Tc(CO)_3$-NSC5 (2) complexes

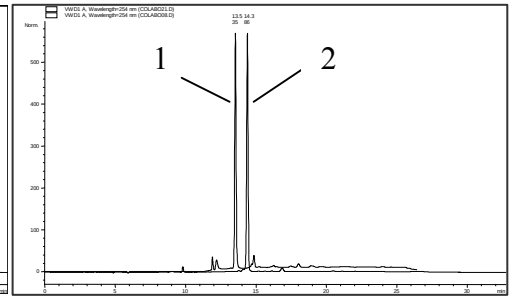


**Fig.2.** UV chromatograms of Re(I)-NSC (1) and Re(I)–NSC5 (2) omplexes

The percentage of $^{99m}Tc(I)$(NSC5) protein binding was around 47%. The results of lipophilicity measurements of examined sample showed that most radioactivity remains in organic phase, thus the distribution coefficient is around 0,66. No change in extractability with pH was observed Biodistribution studies showed minimal organ retention except liver (10.461%/g), intestine (3.012%/g) and kidneys (3.388%/g) of the injected dose at 1 hour p.i.

## Discussion/Conclusion

The studied ligands, having a NSO donor atom set, were easily coordinated with $^{99m}Tc$-tricarbonyl core in aqueous solution forming neutral complexes. Radiochemical purity and yield of labelling were very high. The complexes were very stable for at least 24 hours. HLPC analysis confirmed that these complexes were formed as singular chemical species. Moreover, the derivatization of the imidazolidine ring on the N-1 with an ethyl group did not reduce the labeling efficiency of the ligand as well as the stability of its $^{99m}Tc(I)$ complex. This study showed that these ligands can be derivatized in order to modify the biological behavior by attaching the appropriate biomolecule on the N-1. The labelled NSC5 ligand has been shown to be very stable, and due to its relative lipophilicity has a very good biodiodistribution profile. With these points in mind this chelathing agent provide a promising architecture for use in labelling tumor specific biomolecules.

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