PHYSICAL CHEMISTRY 2012

11th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Under the auspices of the
University of Belgrade

Proceedings

The Conference is dedicated to
Professor Ivan Draganić

September 24-28, 2012
Belgrade, Serbia

ISBN 978-86-82475-27-9 ***Volume*** **1**
ISBN 978-86-82475-28-6 ***Volume*** **II**

Title: PHYSICAL CHEMISTRY 2012 (Proceedings)
Editors: S. Anić and Ž. Čupić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 497; **Format**: B5; Printing finished in September 2012.

Text and Layout: "Jovan"

200- *Coppy printing*

CONTENTS

K-09-P

^{99m}Tc(I)-TRICARBONYL LABELING OF ETHYLENE DIAMINE-N,N´-DI-3-PROPANOATE DIETHYL ESTER AS POTENTIAL RADIOPHARMACEUTICAL AGENT

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Abstract
There is an increasing interest for the ^{99m}Tc labeling of biomolecules by using bifunctional chelating agents like ethylenediamine-N,N´-di-3-propanoate diethyl ester (deeddp). To find new ligand, which can be linked to the small biomolecules and coordinated with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, is a challenging task. Radiolabeling of deeddp with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor, stability studies and biodistribution of formed complexes were carried out, including challenge with histidine. Radiochemical yield of ^{99m}Tc(I)-tricarbonyl-deeddp complexes was higher than 95%. These complexes were stable *in vitro* and showed a very good biological behavior. The radiochemical and biological features of the novel ^{99m}Tc(I)-complexes, as well as, the nature of the ligands, make them very promising candidates for labeling of tumor specific biomolecules.

Introduction
Technetium radiopharmaceuticals, as complexes of the ^{99m}Tc radionuclide, are of great importance in diagnostic nuclear medicine. Over the last few years, the chemistry of a novel organometallic species, $M(CO)_3^+$ (M=Tc, Re), has been intensively developed and the water soluble technetium tricarbonyl complex $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was seen to be very versatile and effective precursor for labeling 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 [2-4].The aim of this study is to label ligand deeddp with ^{99m}Tc(I)-tricarbonyl precursor. The stability of the formed complexes and theirs *in vitro* and *in vivo* properties were investigated too.

Experimental
The sample of ligand was prepared by dissolving in water appropriate amount of substance for obtaining 10^{-3} mol/dm^3 solutions. pH was adjusted to 9.0. ^{99m}Tc-carbonyl precursor was prepared according to the manufacturer instruction (IsoLinkTM, Mallinckrodt Medical B.V., The Netherlands). ^{99m}Tc(I)-tricarbonyl-ligand complexes were prepared by addition of 0.9 ml of ligand solutions to 0.1 ml of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with appropriate pH values. The vial was heated for 30 min in boiling water bath. The labeling efficiency of ^{99m}Tc(I)-tricarbonyl targeted ligand 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. Aliquot of 100 µl of the ^{99m}Tc complexes (final concentration of ligands 10^{-6} M) was added to 900 µl of a 10^{-2} M histidine solution in PBS (phosphate buffered saline), pH 7.4. The samples were incubated at 37^0 C and periodically aliquots were removed and analyzed by HPLC. TCA (trichloroacetic acid) precipitation method for determining the percentage of ^{99m}Tc(I)-tricarbonyl-deeddp bound to proteins (12% human albumin, incubation at 37ºC for different time intervals) was very useful. All lipophilicity measurements were done by solvent extraction method with n-octanol equilibrated with 0.15M 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, 30, 60 and 120 minutes after application of 0.1ml of ^{99m}Tc(I)-tricarbonyl-deeddp. The radioactivity per organ of interest was measured in a NaI(Tl) detector.

Results and Discussion

The bifunctional chelating agent approach is currently among the cutting edge technologies used in the design of new radiopharmaceuticals. The choice of a chelator agent may be crucial in the biological behavior of a radiopharmaceutical.

A novel bifunctional chelating agent deeddp has been synthesized and characterized. Radiolabeling of deeddp with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor with heating at 95ºC and at pH 8-9 led to the formation of ^{99m}Tc(I)-tricarbonyl-coordinated complexes of deeddp with a radiochemical yield higher than 95% as determined by HPLC analysis. The three peaks in the radio-HPLC profile indicated the presence of isomers (Fig.1). Radiochemical stability was monitored during 24 h. ^{99m}Tc(I)-tricarbonyl-deeddp complexes showed a good stability and less than 5% of radiochemical impurities were observed even for the later time point studied. Challenge experiments with up to 1000-fold molar excess of histidine showed no degree of transchelation for radiocomplex during 24 h at 37°C.

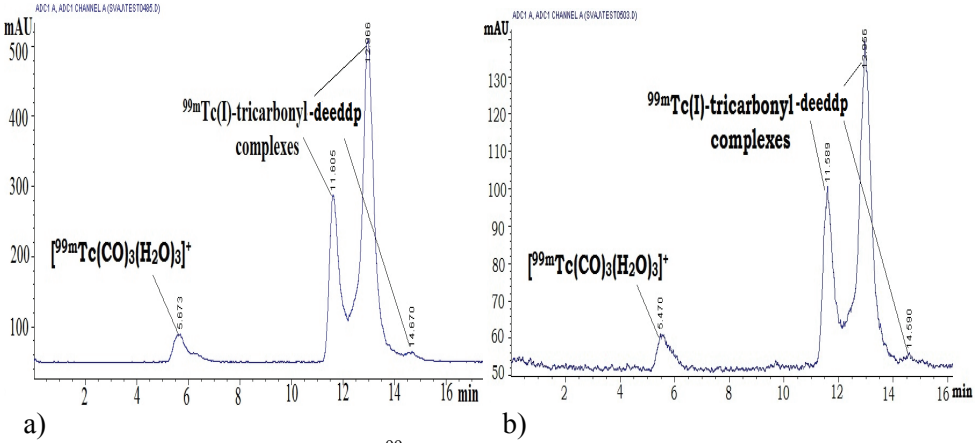

Figure 1. Radiochromatograms of ^{99m}Tc(I)-deeddp complexes a) 30 minutes after labeling b) 24h after labeling.

We assessed the interaction of ^{99m}Tc(I)-tricarbonyl-deeddp complexes with human serum albumin as an important constituent of human blood which could affect on their biological behavior. At 1h, the binding was 10.49±1.23%. The lipo-hydrophilic character of ^{99m}Tc(I)-tricarbonyl-deeddp complexes was evaluated based on the octanol/water partition coefficient (K_d). K_d value was 0.63±0.05 (mean±S.D.) arguing for a higher lypophilic character of the complexes.

Figure 2 shows the biodistribution results for ^{99m}Tc(I)-tricarbonyl-deeddp complexes. The first set of biodistribution data, 5 min post injection (pi), showed a very high uptake in liver, kidneys and intestine. The radioactivity was quickly cleared from liver and kidneys, thereby reaching very low levels within 120 min pi. Moreover, a remarkable intestinal uptake was observed for ^{99m}Tc(I)-tricarbonyl-deeddp complexes even at the later time points studied.

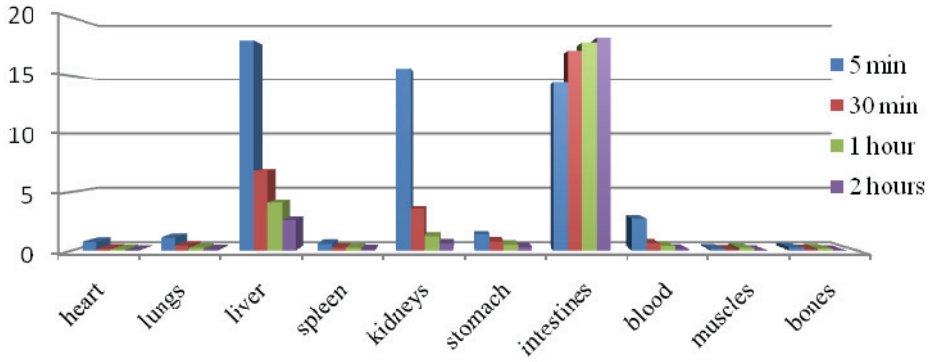

Figure 2. Organ distribution data of ^{99m}Tc(I)- tricarbonyl-deeddp in Health Wistar rats (% ID/g)

Conclusion

The studied ligand, having a bifunctional NN donor atom set, was easily coordinated with ^{99m}Tc(I)-tricarbonyl core in aqueous solution forming neutral complexes. Radiochemical purity and yield of labeling were very high. The complexes were very stable for at least 24 hours. The labeled deeddp ligand has been shown to be very stable against ligand exchange, and due to its relative lipophilicity has a very good biodistribution profile. With these points in mind this chelating agent provides a promising architecture for use in labeling tumor specific biomolecules.

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CIP Volime II

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

544(082)
621.35(082)
66.017/.018(082)

MEĐUNARODNA konferencija iz fundamentalne i primenjene fizičke hemije (11 ; 2012 ; Beograd)
Physical Chemistry 2012 : proceedings. #Vol. #2 / 11th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 24-28, 2012, Belgrade ; [editors S.[Slobodan] Anić and Ž.[Željko] Čupić ; organized by Society of Physical Chemists of Serbia ... et al.]. - Belgrade : Society of Physical Chemists of Serbia, 2012 (Belgrade : Jovan). - VI str., 499-782 str. : ilustr. ; 24 cm

"The Conference is dedicated to Professor Ivan Draganić" --> nasl. str. - Tiraž 200. - Bibliografija uz svaki rad. - Registar.

ISBN 978-86-82475-28-6
1. Društvo fizikohemičara Srbije (Beograd)
a) Физичка хемија - Зборници b) Електрохемијско инжењерство - Зборници c) Наука о материјалима - Зборници
COBISS.SR-ID 193433356