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# Blood plasma model predictions for the proposed bone-seeking radiopharmaceutical [$^{117m}$Sn]Sn(IV)-N,N′,N′-trimethylenephosphonate-poly(ethyleneimine)

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ABSTRACT

In an attempt to elucidate the *in vivo* stability of the prospective radiopharmaceutical [$^{117m}$Sn]Sn(IV)-PEI-MP, where PEI-MP stands for N,N′,N′-trimethylenephosphonate-polyethyleneimine, glass electrode potentiometry was used to determine the stability constants of the $Sn^{4+}$ ion as complexed with a variety of physiological amino acids. In addition, linear free energy relationship (LFER) correlation plots were used to extrapolate the constants of the major blood plasma ligands, based on data from $Cu^{2+}$, $Pb^{2+}$, and $Zn^{2+}$. In so doing, a thermodynamic model of blood plasma was established for $Sn^{4+}$ from which the complexation tendencies of $Sn^{4+}$ were predicted in the event of the intravenous administration of such a drug. It was found that the Sn(IV)-PEI-MP could succumb to competition by the glutamine amino acid, which forms more stable complex(es), whilst the PEI-MP gets taken up largely by $Ca^{2+}$. Also, this study shows the value of the *in vitro* experiments and modeling performed for radiopharmaceutical research and for attempts to reduce the number of animal experiments.



## 1. Introduction

In the ongoing development of anti-cancer radiodrugs, selective targeting and high linear energy transfer (LET) are of the foremost criteria for achieving efficacy [1]. One of the most elusive forms of cancer is that of metastasis in bone – which is tumours that develop as the result of proliferation of malignant growths in organs remote from that of the source (e.g. breast and prostate cancer) – a disease commonly responsible for severe pain, and negating treatment of the primary cancer [2–5].

The most widely prescribed treatment for bone ailments is that of bisphosphonate therapy, which includes the use of drugs such as, among others, pamidronate, i.e. 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD) [6,7] and etidronate, 1-hydroxyethylene-diphosphonate (HEDP) [8]. Novel radiopharmaceuticals for the palliation of bone pain have been proposed, which comprises of radiolabelled tin, namely [$^{117m}$Sn]Sn(II) or [$^{117m}$Sn]Sn(IV) [9–11].

$^{117m}$Sn decays by the emission of conversion electrons and a photon (159 keV, 86.4%), with a half life of 13.6 days. In tissue the electrons have been reported to have a short range/trajectory of about 0.2–0.3 mm [9], which is paramount in the treatment of bone for minimizing radiotoxicity to the sensitive bone marrow. Furthermore, the photon affords an additional benefit by enabling *in vivo* imaging of the distribution of the drug.

In order to exploit these characteristics, effective tumour targeting is very important, therefore modified phosphonates are proposed, for example PEI-MP, to selectively deliver the radionuclide to the site [12] – Fig. 1. The PEI-MP has an advantage over conventional bisphosphonate treatments as it can exploit the phenomenon known as the enhanced permeation and retention (EPR) effect whereby macromolecules accumulate within tumours [13], thereby increasing the lesion to healthy tissue ratio. In consideration of the viability of such drugs there are some fundamental issues that need to be addressed, such as the stability of the agent or it's components *in vivo*.

To determine the stability of metal–ligand complexes, glass electrode potentiometry can be used to measure the stability constants ($\beta$). In this paper, the stability of the complexation of $Sn^{4+}$ with physiological ligands was studied. A selection of 11 amino acids was made from the many potential physiological ligands to

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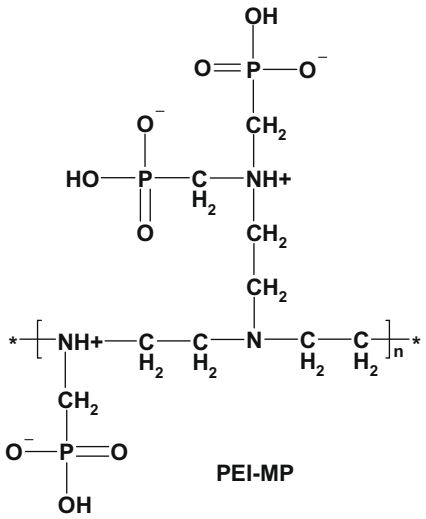


**Fig. 1.** Molecular structure of N,N′,N′-trimethylenephosphonate-polyethyleneimine (PEI-MP), represented in an arbitrary protonation state.

construct LFER curves [12]. From these LFER's the stability constants of the complexation of $Sn^{4+}$ with the remaining amino acids or other blood plasma ligands could be estimated based on the known data for the complexes of $Cu^{2+}$, $Pb^{2+}$ and $Zn^{2+}$. In so doing the blood plasma distribution and fate of the components of the proposed radiopharmaceutical comprised of $[Sn]Sn^{4+}$ complexed with PEI-MP- hence $Sn^{4+}$-PEI-MP – was predicted.

## 2. Materials and methods

### 2.1. Reagents and apparatus

All reagents were of analytical grade and obtained from Merck, KGaA, Darmstadt, Germany and Sigma–Aldrich. Potentiometric titrations were carried out using a Radiometer TitraLab dual burette titration workstation and the data automatically captured with TitraMaster 85 data collector software. The titrations were performed at constant temperature of 25.0 ± 0.1 °C, with the aid of a jacketed vessel through which water was circulated from Grant W12 thermostated bath.

The temperature of 25.0 °C was chosen so as to be consistent with the majority of formation constants reported in literature and in the Evaluation of Constituent Concentrations in Large Equilibrium Systems (ECCLES) database especially [14], which was used to as a simulation of blood plasma so as to construct a blood plasma model for $Sn^{4+}$. The difference between formation constants obtained at 25.0 °C and at 37.0 °C is negligible, as demonstrated by Zeevaart et al. [10,11] in their comparison of blood plasma model predictions with the biodistribution in rodents, for N, N-dimethylenephosphonate-1-hydroxy-3-aminopropylidenediphosphonate (APDDMP) as complexed with $[^{117m}Sn]Sn^{2+}$. Furthermore, a temperature of 25.0 °C is easier to maintain constant and minimizes evaporation and condensation within the reaction vessel, which could affect the concentration of the titre. The electrode used was a combination glass electrode (Ag/AgCl, 3 M KCl reference), namely the LL-Unitrode (60259100) by Metrohm. The electrode was calibrated daily by means of a strong acid–base titration [12]. The standard electrode potential and HCl concentration were determined daily.

All pipettes used during the experiments were calibrated by weighing the respective volumes of demineralised water, and the corrected experimental volumes were used throughout data analysis and modeling process. All solutions were prepared with a total ionic strength ($\mu$) of 0.15 M so as to simulate physiological blood plasma conditions. Stock solutions of the amino acids were prepared at a concentration of 0.1 M and made up with a solution of 0.15 M NaCl. The ligands were: cysteinate, histidinate, aspartate, glycinate, serinate, ascorbate, citrate, lactate, malate, oxalate, salicylate, succinate, tartrate, L(+)-alaninate, glutaminate and asparaginate.

Titrations were performed at various concentrations of the respective ligand relative to that of $Sn^{4+}$, with the ligand in excess so as to avoid hydrolysis and precipitation of the tin. The following ligand-to-metal ion ratios were used: 2:1, 2.5:1, 3:1, and 4:1, unless otherwise specified, with the $Sn^{4+}$ concentration of 1.67 mM. The $SnCl_4{\cdot}5H_2O$ salt was used and weighed directly into the reaction vessel. The tin was not prepared in a standard solution due to complications with hydrolysis, and inconsistent standardization results. Prior to each titration the solution mixture of $Sn^{4+}$ with the respective ligand were acidified with 0.1 M HCl (in 0.05 M NaCl) to yield an initial acid concentration ($[H^+]_{vessel}$) of 8.40 mM. Using a 0.15 M NaCl solution the reaction mixtures were made up to a fixed initial volume of 30 ml in the jacketed vessel. With constant stirring the respective solutions of $Sn^{4+}$ and amino acid were titrated against the titrand 0.05 M NaOH (in 0.1 M NaCl), which was prepared using 0.1 M NaOH Titrosol solution, and standardized against potassium hydrogen phthalate (KHP). Effectively, the $[Na^+]$ concentration was kept constant for throughout the titrations. The acid dissociation constants for each amino acid were also determined under similar conditions, in the absence of $Sn^{4+}$. Three titrations with varying concentrations of the ligand within the reaction vessel, namely 1.4 mM, 2.8 mM and 4.2 mM, and these were acidified to an initial acid concentration of 14.5 mM HCl. The same procedure was adopted for the determination of the stability of the complexes of $Sn^{4+}$ with PEI-MP, as well as the proton dissociation constants. All titrations of PEI-MP were performed using the size fraction of 10–30 kDa.

### 2.2. Speciation modeling

The titration data were imported into the equilibrium simulation by titration analysis (ESTA) modeling program [15] where the stability constants were calculated and refined. Hydrolysis constants for $Sn^{4+}$ were obtained from literature [16] and included in the models: $\log \beta_{10\text{-}1} = -1.94$; $\log \beta_{10\text{-}6} = -24.11$ (where $\beta_{10\text{-}1}$ refers to the overall formation constant of the complex comprising of 1 metal ion, 0 ligand and −1 proton). Upon completion of suitable $Sn^{4+}$ complexation speciation models for the selected amino acids, the constants were then compared against the respective data for complexes of $Cu^{2+}$, $Pb^{2+}$ and $Zn^{2+}$. LFER curves were then established by plotting the values of the stability constants of the ML species ($\log \beta_{110}$) of $Sn^{4+}$ against the $\log \beta$ for the same species of Cu, Pb and Zn for each ligand, as illustrated by Zeevaart et al. [12]. This provided three curves from which the remaining *in vivo* ligands could be extrapolated, and estimated as the average of the three. Ligands were selected from the above list – of those that had been measured by potentiometry – for which their formation constants for the ML complexes ($\log \beta_{110}$) were in close agreement. These were then used to construct the LFER curves, namely: aspartate, serinate, glycinate, citrate, lactate, malate, oxalate, salicylate, succinate, histidinate and tartrate. For all the speciation models of $Sn^{4+}$measured, none of them possessed the species ML, therefore a $\log \beta_{110}$ value was calculated from the respective ligand dissociation constants and the $Sn^{4+}$ hydrolysis constant [16].

### 2.3. Blood plasma simulation using ECCLES

All the formation constants that had been measured – by glass electrode potentiometry, and estimated – by LFER correlations,