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A comparative thermodynamic study of the formation of Scandium Complexes with DTPA and DOTA.

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The complexation of scandium (III) by various polyaminopolycarboxylic ligands (DTPA and DOTA) was studied by capillary electrophoresis with ICP-MS detection in 0.1 mol L−1 NaCl ionic strength solutions at 25°C. The results confirmed the formation of the 1:1 complexes for Sc(III)-DOTA and Sc(III)-DTPA systems. For each complex, the thermodynamic conditional constant was determined from the experimental data. The thermodynamic constants were extrapolated to zero ionic strength using Davies equation and then compared to previously published data. These results were compared with free-ion selective radiotracer extraction (FISRE) data, which is a suitable method at trace concentrations. The relative order of stability constants was preserved; as this method is experimentally simple, it is suitable for quick relative comparison of stability constants values under trace concentrations.

1- Introduction

On the way to a personalized medicine, nuclear medicine offers both diagnostic tools and therapeutic drugs utilizing various radioisotopes. Until recently most radiopharmaceuticals were designed to be used solely for either diagnostics or therapeutics. Currently radionuclides used for imaging, such as ^{68}Ga or ^{111}In, are different from that used for therapy: ^{90}Y or ^{177}Lu. One radionuclide would be used to image individual patient disease states and evaluate their receptor expression, metabolic rate, clearance and handling, and a second radionuclide would be used for therapy. The problem with this approach arose due to differences in the chemistry of the radionuclides themselves, which have been shown to affect the overall distribution and mechanism of localization, resulting in an over- or underestimation of dose to critical tissues and the dose being outside the optimum range of efficacy [1]. Using the same metal to perform both the diagnosis and therapy would result in a better determination of the absorbed dose to the dose limiting organs and would give a better indication of the therapeutic activity to administer. Such radiopharmaceutical pair utilizing diagnostic and therapeutic radioisotopes is called “theranostics” [1].

Among the radionuclides available, there is significant interest in therapeutic radioisotope ^{47}Sc (β^-, $\tau_{½}$ 3.35 d, E_β 0.143 and 0.204 MeV with 68 and 32%; γ, E_γ 159.4 keV, 68%) as it matches with positron emitting ^{44}Sc (β^+, $\tau_{½}$ 3.97 h, E_β 0.63 MeV, 94.3%) or ^{43}Sc (β^+, $\tau_{½}$ 3.89 h, E_β 0.344 MeV and 0.508 MeV, 17.2 and 70.9% respectively) forming an ideal theranostic pair. Potential of ^{47}Sc for nuclear medicine has been already investigated [2-4]. Due to its soft positron emission, ^{44}Sc is very suitable for PET imaging. Its half-life is perfectly matching pharmacokinetics of oligopeptides, with a better $\tau_{½}$ in comparison to ^{68}Ga ($T_{1/2}$ = 68 min). In addition, the radioisotope can be produced together with its long-lived isomeric exited nucleus, ^{44m}Sc (γ, $\tau_{½}$ 2.44 d, 98.8 %, E_γ 270.9 keV), decaying to ^{44}Sc with the soft γ emission. The third γ ray is suitable for three-photon coincidence imaging which may further increase resolution of the current PET imaging [5]. Half-life of ^{44m}Sc is matching *in vivo* pharmacokinetics of antibodies and, due to its low-energy transition (recoil energy only 0.89 eV), it can serve as *in vivo* generator of the PET radioisotope ^{44}Sc as the daughter ^{44}Sc stays inside chelator after decay of the parent ^{44m}Sc [6]. The theranostically matched therapeutic radionuclide ^{47}Sc has rather soft β^- emission suitable for treatment of cancer metastases and also soft γ-emission which is very similar to that of ^{99m}Tc (the most commonly used radionuclide) and, thus, ideal for currently used SPECT cameras. The ^{44m}Sc and ^{47}Sc have similar half-lives very suitable for radiopharmaceuticals with antibodies or their fragments and, thus, they form a unique and very promising theranostic pair for cancer treatments. Utilizations of the theranostic pair can be spread from targeted radioimmunotherapy (^{44m}Sc/^{47}Sc pair with antibodies) to treatments with labeled oligopeptides or small molecules (^{44}Sc/^{47}Sc pair). Recently, the scandium chemistry has revealed a growing

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Electronic Supplementary Information (ESI) available: Experimental details on electrophoeretic mobilities

interest with an increasing number of papers available on scandium: from $^{44}Ti/^{44}Sc$ generator [7-8], cyclotron produced $^{44m}Sc/^{44}Sc$ [9-10], ^{45}Sc [11], ^{46}Sc [12-13] or ^{47}Sc [3,4,14]. The scandium radioisotopes have become to be more easily available in the recent years. The ^{44}Sc can be produced by generator employing ^{44}Ti as a long-lived parent radioisotope [7, 15]. On the other hand, the radioisotope can be also produced in most of medical cyclotrons designed for ^{18}F production and, during this mode of production; ^{44m}Sc is also prepared in a mixture with ^{44}Sc. The ARRONAX cyclotron produces also $^{44}Sc/^{44m}Sc$ from enriched $^{44}CaCO_3$ target via the deuteron production route [10].

To be used for imaging and, more importantly, for targeted radiotherapy, metallic radioisotopes must be tightly bound in a complex to avoid non-specific deposition of their "free" form and to ensure elimination of unchanged conjugate out from body if not delivered to target organ/tissue. Mostly, these complexes must exhibit a high thermodynamic stability and kinetic inertness. In addition, the ligands have to manifest a fast complexation of the metallic radioisotopes even in highly diluted solutions, a high selectivity for the particular metal ion as well as an ability to be conjugated to a biological vector molecule (bifunctional ligands). A number of reviews have shown that design of new radiopharmaceuticals is viable multidisciplinary field involving physics, chemistry, biology and medicine [16-22].

As the rare-earth element, scandium is generally considered as a cousin of lanthanides and, similarly, scandium is almost exclusively present in its compounds in trivalent state. However, chemistry of trivalent scandium has some differences; it is smaller (having more hard character and higher preference for hard oxygen donor ligands) and prefers donor numbers from six to eight. Still, chemistry of trivalent scandium is much less developed than that of trivalent lanthanides [24]. For medical application of scandium radioisotopes, multidentate ligands already used in Gd(III)-based MRI contrast agents as well as for radiolanthanides, i.e. derivatives of DTPA (DTPA = diethylenetriamine-*N,N,N′,N″,N‴*-pentaacetic acid) or DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), are the first choice. It has been shown that DOTA and DTPA derivatives are suitable ligands for scandium radioisotopes [6, 24]. Their oligopeptide [9, 15, 25-26], antibody [13] or other conjugates [27-28] have been investigated for complexation of the scandium radionuclides. Recently, we have investigated chemistry of Sc(III)–DTPA and Sc(III)-DOTA complexes in details [29-30]. The study confirms that DOTA is suitable chelator for trivalent scandium; the thermodynamically very stable complex is formed rather quickly and is kinetically inert. Thermodynamic data for scandium (III) complexes with polydentate ligands are scarce; only some stability constant data have been published for Sc(III) complexes of DTPA [12, 31] and DOTA [24]. Stability constants were determined for both complexes by combination of potentiometry and NMR spectrometry [30]. Nonetheless, discrepancies have been noticed for the Sc(III)-DOTA or DTPA systems depending upon the method used for the determination of the stability constant (i.e. Free Ion Selective Radiotracer Extraction –FISRE, or potentiometry). For instance, stability constant values have been reported for FISRE to be 22 and 22.5 for DTPA and DOTA respectively [1]2 whereas more recently, the stability constants, log K_{ScL} determined by potentiometry were 27.43 and 30.79, for DTPA and DOTA complexes, respectively [30]. These values are several orders of magnitude higher than those of lanthanide (III) complexes of the same ligands. The methods have to be combined as potentiometry itself led to misleading results due to quantitative complex formation at pH below 1.5, thus, out of potentiometry pH range. In addition for Sc(III)-DOTA system, slow complex formation complicated the measurements and *out-of-cell* titration method was used [32]. Whereas, FISRE was based on competition of chelating resin and ligand in solution for a metal ion, it could give an access to the conditional thermodynamic equilibrium constant under non-ideal conditions. The ligand-metal ion stability constant can be determined through analysis of efficiency of the competition as a function of the parameters affecting the complexation, i.e. concentration/excess of the ligand or pH. Thus, stability constants could be estimated by a fitting dependence of the distribution coefficient of Sc(III) between the resin and supernatant, K_d, on the ligand concentration in the supernatant.

Nonetheless, some discrepancies have been noticed for the same complexes depending on the scale used (i.e. macroscopic concentrations or trace concentrations) and the methodology used for the constant determination (i.e. potentiometric titration or FISRE). Those discrepancies are against all thermodynamic principles and must be clearly established.

A quite recent work has examined the formation of trivalent actinides complexes with DTPA using the coupling between Capillary Electrophoresis (CE) and ICP-MS [33]. So the motivation of this work was to examine through CE-ICP-MS, the constant of formation of scandium-complexes. We take the advantage of the hyphenated technique between capillary electrophoresis and ICP-MS to carry out direct speciation measurement at tracer scale. This method does not provide a complexation coefficient by opposition to potentiometric titrations or Free Ion Selective Radiotracer Extraction (FISRE) method. A discussion will be done on the methodology used for the determination of the stability constant of metal complexes. Additional work has been performed with FISRE method for implementing this discussion.

2- Material and methods

2.1. Chemicals

A 0.1 mol L^{-1} NaOH solution (VWR, Titrinorm) was used to precondition the capillaries (described in the next following section). N,N-Dimethylformamide (DMF) (Sigma, 99%) was added to the samples as a neutral UV active compound to measure the electroosmotic flow. All the solutions were filtered through 0.45 μm nylon filters (Nalgene, Rochester, NY) and were degassed (sonicated for 10 min) prior to be used in capillary electrophoresis. The

capillary was submitted to liquid thermostating (coolant, Beckman Coulter). DMF aliquot was added in each sample and was monitored by UV at 214nm for neutral species. HCl solution was purchased from Sigma. Chelex-100 resin (Biorad) was previously washed with HCl 6 mol.L^{-1} (Prolabo Normapur, 70%) to remove potential impurities then conditioned with the aqueous solution before to be used. The mass of resin for each sample was chosen to minimize the uncertainties on distribution coefficient.

Deionised water (Millipore Alpha-Q, 18.2MΩcm) was used throughout the experiments. Background electrolytes (BGE) and different samples were prepared from weighted amounts of NaCl (Acros Organics, ≥99%), $ScCl_3$ (Perkin), diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA) (Aldrich) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) (Chematech). The pH of different samples was adjusted by adding either HCl (Prolabo Normapur, 70%) or NaOH (Prolabo Normapur, 99%) solutions. pH was monitored along the time in order to ensure its stability before any further analysis.

Scandium standard solution (Solution Plasma CAL-Scandium, 10000µg/mL in HNO_3 4%) was purchased from SCP-Sciences and diluted solution (10^{-6}M to 10^{-2}M) were freshly prepared for ICP-MS calibration purpose. In the meanwhile, ICP-AES ICAP 6500 DUO (Thermoscientific) was used to determine scandium concentration at 361.3 nm in supernatant of batches experiment at equilibrium in FISRE experiments.

In the present work, the H^+ concentrations have been measured, i.e. pcH, but it was assumed that H^+ activity scale and the H^+ concentration scale were similar due to the low concentrations used. So in the following, pH will stand for pcH.

2.2. Electrophoresis capillary – ICP mass spectrometry device

A Beckman Coulter P/ACE MDQ commercial Capillary Electrophoresis (CE) system equipped with an UV detector (Fullerton, USA) was used for all the measurements. The measurements were carried out using conventional fused silica capillaries, 100 μm internal diameter, 69.9 cm total length (Beckman Coulter, Fullerton, USA). The capillaries were preconditioned by rinsing (1) with deionised water, (2) with a 0.1mol L^{-1} NaOH solution, (3) with a 0.1 mol.L^{-1} HCl solution, (4) with deionized water again and finally (5) with the adequate BGE before use (at 5 psi during 5min for each solution).

The CE system was provided with a tailor-made capillary cartridge support designed for the adaptation of an external detector, *i.e.* an ICP-MS. Indeed, two detectors were used: a UV conventional detector for the measurement of the electroosmotic flow by means of the neutral DMF U.V. active compound at 214 nm, and a mass spectrometer detector for the measurement of the effective mobilities of scandium. Samples were injected hydrodynamically and detected with the UV detector placed 10.2 cm from the injection point. The UV spectrophotometric signal was collected by the capillary electrophoresis software (Karat 5.0) whereas the transient mass spectrometry signals were acquired by the axiom software (PlasmaLab). Isotopic measurements were carried out using an Axiom (VG Elemental, Winsford, Cheshire, UK) inductively coupled plasma sector field mass spectrometer (ICP-SF-MS). A commercial parallel path micro-nebulizer (Mira Mist CE, Burgener Research Inc., Mississauga, Ontario, Canada) was used. A make-up liquid (HNO_3 2% and ethyl alcohol absolute 10%) is injected in the parallel path nebulizer in order (i) to improve the signal stability by decreasing the surface tension of the water droplets and the size of the droplets and (ii) to provide the nominal flow rate for the nebulizer. The make-up solution was introduced by a syringe pump (11 Pico Plus, Harvard Apparatus, Holliston, Massachusetts, USA) at the nominal flow rate 7 μLmin^{-1}. The nebulizer was connected to a borosilicate spray chamber (mini glass chamber +0.5" ball joint adapter, Burgener). The ICP-MS operated in the medium resolution mode (R = 2957) to avoid SiOH interference. The fast scanning magnet of the mass spectrometer allowed acquiring sharp and narrow CE signals. ^{45}Sc isotope was selected for analysis and the exact mass scanned was 44.9403 in order to discriminate from SiOH specie (mass at 44.9648).

The pHs of all the solutions and BGEs were controlled in order to ensure their stability around defined pH (1.44; 2.5; 3.2 for DOTA and 1.35; 2.4 for DTPA). 0.2 μL of pure DMF was added to the sample as a reference. t_{BGE} is 3s and ΔP_{BGE} is 0.3 psi for all injections. The voltage was varied from 4kV to 6kV depending on the pH of the solution analyzed. It was preliminary checked that the Ohm law was verified for the capillary in all these conditions. The voltage was fixed at 4kV for solutions at pH = 1.3 and 1.4; 5kV for solutions at pH = 2.4 and 2.5; and 6kV for pH = 3.2 respectively. Before each change in the experimental conditions, the capillary was rinsed with 0.1 mol.L^{-1} solution of NaCl at the desired pH value.

The pH of each BGE was measured before and after the separation using a "high-precision 780 pH meter" (Metrohm) and a "combined metrosensor glass electrode" named "biotrode" (Metrohm). The pH variations were less than 0.3 pH unit resulting in a negligible variation of ligand concentration during the separation. The calibration of the electrode was carried out daily using commercial solutions (pH 1.68, 6.86 and 9.18 ITT Analytics).

2.3. FISRE method: Distribution coefficient between two non-miscible phases

This method has been employed to determine the stability constants [12] at the tracer level using a Chelex-100 cationic exchange resin, conditioned in a preliminary step. This chelating resin competes with the ligands for Sc^{3+} ion and the speciation is determined by modelling solid/liquid separation. A mass of the chelating resin was mixed with bulk solution of ^{45}Sc at a concentration of 5.10^{-6} M of the isotope. The ligands stock solution (at 10^{-2}M) was added to reach final ligand concentration ranging from 10^{-7} to 10^{-3} M, to get optimized solid-to-liquid ratios (S/L) = 3 g.dm^{-3}. All the measurements were performed at ionic strength I = 0.1 mol.dm^{-3} of NaCl solution for a total volume of each sample of 4mL. The pH of

the suspension was adjusted to the desired pH value depending on the ligand studied (DOTA, DTPA respectively). These pH values were considered taking into account the stability constants values (log K_{ScL}) obtained from the equilibrium data. As the distribution coefficients were calculated as a function of dried resin mass, the percentage of humidity was determined by placing 5 samples of each pre-conditioned resin in oven at 105°C for one day. The final distribution coefficients were calculated based on the arithmetic averages of replicate analysis. The resin concentration (in g/mL) was chosen for each batch to minimize the global uncertainty of partition coefficient. The reproducibility of logarithm of K_D was better than 5%.

The different samples were agitated for at least one week to reach equilibrium. The suspension was then filtered with 0.22 µm cellulose acetate (Millipore). The first milliliter of filtrate was discarded to prevent from potential adsorption of filters and the following ones were kept for ICP-OES analysis after dilution in HNO_3 1%. Scandium concentrations were determined in the resulting suspension and an experimental K_d is plotted as a function of the quantity of the total ligand concentration introduced (which is in large excess in comparison to the initial concentration of scandium).

3. Data treatment

The ligand protonation constants determined previously [12] or more recently [30, 33] are used in this calculation as constant parameters and data could be refined with slightly changed protonation constants of ligands if necessary. For DTPA, these constants were extracted from Leguay et al. [33] based on a review from literature, whereas for DOTA, they have been redetermined by Pniok et al. [30] in the same conditions (I=0.1M).

3.1. CE-ICP-MS

The scandium electrophoretic mobility (μ_{ep}) was calculated (Equation 16) by subtracting the osmotic mobility (μ_{eo}) measured with DMF to the scandium apparent mobility (μ_{app}):

$$\boldsymbol{\mu_{ep} = \mu_{app} - \mu_{eo} = \frac{L_t^2}{V}\left(\frac{1}{t_{app}} - \frac{1}{t_{eo}}\right)} \quad \text{Eq. 1}$$

with L_t the capillary total length (cm), l the distance between the U.V. and the negative side of the capillary (cm), V the applied voltage (V), t_{app} the migration time of scandium (s) and t_{eo} the migration time of DMF (s).

For fast equilibrium reaction, rapid and permanent exchanges during the separation occur between the metal and the ligand. Therefore, the measurement requires the presence of a constant concentration of ligand into BGEs. In practice, only a single peak is observed for which an average apparent mobility can be calculated:

$$\boldsymbol{\mu_{app} = \sum_i(\alpha_i \mu_i) + \mu_{eo}} \quad \text{Eq. 2}$$

Where μ_i refers to the mobility of the scandium species i, and α_i refers to its corresponding molar fractions. Using the mass balance equations and the law of mass action, Equation (2) can be rearranged into Equation (3):

$$\boldsymbol{\mu_{app} = \frac{\mu_{Sc^{3+}} + \sum_{i=1}^{j} \beta_{ScH_iL}[L^{p-}][H^+]^i \mu_{ScH_iL}}{1 + \sum_{i=1}^{j} \beta_{ScH_iL}[L^{p-}][H^+]^i} + \mu_{eo}} \quad \text{Eq. 3}$$

For slow equilibrium reaction as regard of migration time, the proportion of different scandium species can be considered as constant. Multiple peaks are observed as a function of simultaneously separated species. The area of each peak is directly linked to each species concentration.

As experimental data were obtained in acidic media for low total scandium concentration, we can assume that aquo ion Sc^{3+} are the major species in the absence of ligands. From Eq. 3, the percentage of each species can be described as:

$$\boldsymbol{\frac{[ScH_iL^{3+i-p}]}{[Sc(III)]} = \frac{1}{1 + \frac{1}{\beta_{ScH_iL}[L^{p-}][H^+]^i}}} \quad \text{Eq. 4}$$

$$\frac{[Sc^{3+}]}{[Sc(III)]} = \frac{1}{1 + \beta_{ScH_iL}[L^{p-}][H^+]^i} \quad \text{Eq. 5}$$

The stability constant of the reaction can also be deduced from the intercept of both curves:

$$\log \beta_{ScH_iL} = -\log[L^{p-}][H^+]^i \quad \text{Eq. 6}$$

In both techniques, we can conclude that we can determine the complex stoichiometry only by studying the system as a function of pH. For a defined pH, considering that protonation reaction of a complex is a fast equilibrium, the apparent constant can be determined as followed:

$$\boldsymbol{log\beta_{app} = log\ \sum_i \beta_{ScHiL}\,[H^+]^i} \quad \text{Eq. 7}$$

3.2. FISRE method

Scandium is adsorbed onto a Chelex-100 chelating resin through a complexation on iminodiacetate groups. The reaction can be simplified by considering only the exchange between the protons from the carboxylic groups (–XH) and the aquo ion of scandium (Eq. (8)).

$$Sc^{3+} + 3\overline{HX} \rightleftharpoons \overline{X_3Sc} + 3H^+ \quad \text{Eq. 8}$$

Where the overlined species represent the species adsorbed onto the solid phase. The associated apparent extraction constant can thus be expressed as:

$$K_{ads} = \frac{[H^+]^3[\overline{X_3Sc}]}{[Sc^{3+}][\overline{XH}]^3} \quad \text{Eq. 9}$$

As scandium was used as trace concentration with regard of the exchange capacity of resin (Ce), $[\overline{XH}]$ can be approximated to Ce. Moreover, the studies were performed in acidic media where no hydroxide complexes of scandium are significant species. Then, using the mass balance equations and the law of mass action, we obtained the following system:

$$K_{ads} = \frac{[H^+]^3[\overline{X_3Sc}]}{[Sc^{3+}]C_e{}^3} \quad \text{Eq. 10}$$

The experimental distribution coefficient is defined as the total adsorbed scandium concentration $[\overline{X_3Sc}]$ (in mol/kg) over the concentration of aqueous scandium $[Sc(III)]_{sol}$ remaining in solution and determined from initial concentration of Sc(III) $[Sc(III)]_{tot}$:

$$K_d = \frac{[\overline{X_3Sc}]}{[Sc(III)]_{sol}} = \frac{[Sc(III)]_{tot}-[Sc(III)]_{sol}}{[Sc(III)]_{sol}}\frac{V}{m} \quad \text{Eq. 11}$$

Where *V* and *m* are the volume of aqueous phase and the mass of dried resin, respectively.
From Eq. 10 and Eq. 11, the theoretical expression of distribution coefficient can be determined as:

$$K_d = \frac{[\overline{X_3Sc}]}{[Sc(III)]_{sol}} = \frac{[\overline{X_3Sc}]}{[Sc^{3+}]\cdot\alpha_{Sc^{3+}}} = K_{ads}\frac{C_e^3}{[H^+]^3\cdot\alpha_{Sc^{3+}}} \quad \text{Eq. 12}$$

With $\alpha_{Sc^{3+}}$ the complexation coefficient of free aquo ion Sc^{+3}.

The behavior of scandium in different systems is influenced by the presence in solution of DTPA or DOTA ligands (L) due to the formation of the associated complexes. Considering the apparent complex formation reaction and its associated thermodynamic constant (Eq.(13) and Eq.(14)):

$$Sc^{3+} + L^{p-} + iH^+ \rightleftharpoons ScH_iL^{3-p+i} \quad \text{Eq. 13}$$

$$\beta_{ScH_iL} = \frac{[ScH_iL^{3-p+i}]}{[Sc^{3+}][L^{p-}][H^+]^i} \quad \text{Eq. 14}$$

The complexation coefficient can also be explained as:

$$\alpha_{Sc^{3+}} = \frac{[Sc(III)]_{sol}}{[Sc^{3+}]} = \frac{[Sc^{3+}]+\sum_{i=j}^{4}[Sc(OH)_j^{3-i}]+\sum_i[ScH_iL]}{[Sc^{3+}]} \quad \text{Eq. 15}$$

The hydrolysed species of scandium have been taken into account in all calculations. Their contribution is minor and could be neglected. The following equilibria with the corresponding log K values were used [34].

$Sc^{3+} + H_2O \rightleftharpoons [Sc(OH)]^{2+} + H^+$ log *K* = – 4.3
$[Sc(OH)]^{2+} + H_2O \rightleftharpoons [Sc(OH)_2]^{+} + H^+$ log *K* = – 9.7
$[Sc(OH)_2]^{+} + H_2O \rightleftharpoons [Sc(OH)_3] + H^+$ log *K* = –18.1
$[Sc(OH)_3] + H_2O \rightleftharpoons [Sc(OH)_4]^{-} + H^+$ log *K* = –26

For low concentration of ligand, no significant complexation occurs, thus $[ScH_iL]$ can be neglected and Eq. 15 can be simplified as:

$$\alpha_{Sc^{3+}} = \frac{[Sc^{3+}]+\sum_{i=j}^{4}[Sc(OH)_j^{3-i}]}{[Sc^{3+}]} = 1 + \sum_j \frac{\beta_j}{[H^+]^j} \quad \text{Eq. 16}$$

Where β_j is the thermodynamic constant of hydrolysis reactions.
Thus, from Eq.12 when the pH is fixed, K_d is constant and equal to:

$$K_d = K_{ads}\frac{C_e^3}{[H^+]^3\cdot\left(1+\sum_j\frac{\beta_j}{[H^+]^j}\right)} \quad \text{Eq. 17}$$

Where K_d is distribution coefficient between the resin and supernatant, K_{ads} the equilibrium constant for binding scandium (III) to the resin.
Moreover, when the ligand complex becomes the major scandium species, Eq. 15 becomes:

$$\alpha_{Sc^{3+}} = \frac{\sum_i[ScH_iL]}{[Sc^{3+}]} \quad \text{Eq. 18}$$

Thus, Eq. 18 could be rearranged as:

$$\alpha_{Sc^{3+}} = \sum_{i=1}^{j}\beta_{ScH_iL}[L^{p-}][H^+]^i \quad \text{Eq. 19}$$

where $[L^{p-}]$ is the concentration of the free ligand in its basic form. The concentration ligand at any pH is calculated according to the equation (12).

$$[L^{p-}] = \frac{C_t}{1+\sum_{i=1}^{p}\left(\prod_{j=i}^{p}\frac{[H^+]}{K_{a_j}}\right)} \quad \text{Eq. 20}$$

where C_t is the total aqueous concentration of ligands, K_{a_j} is the thermodynamic protonation constant *j* of the ligand, defined as:

$$K_{a_j} = \frac{[H_{j-1}L^{j-1-p}][H^+]}{[H_jL^{j-p}]} \quad \text{Eq. 21}$$

If the ligands concentration is significantly higher than the initial scandium concentration, the free ligand concentration is assumed to be equal to the initial ligand concentration and Eq 12 can be rearranged as:

$$logK_d = log\sum_i\frac{K_{ads}C_e^3}{\beta_{ScH_iL}[H^+]^{3+i}} - log[L^{p-}] \quad \text{Eq. 22}$$

To conclude, by representing the distribution coefficient as a function of the total ligand concentration for a constant pH value, a constant value could be observed for low concentration and a slope equal to 1 for higher concentrations. The intercept of both straight lines allows us to determine the apparent complexation constant (see eq. 7).

4. Results

Since the interaction of actinides with DTPA exhibit high stability constant values, they were studied by capillary electrophoresis and ICP-MS (CE-ICP-MS). Thus, it seemed suitable to examine and establish through this technique a complete set of thermodynamical data on scandium-polyaminopolycarboxylate ligands. In addition, CE-ICP-MS allows a direct access to the speciation and not to a partition coefficient like in the case of potentiometry or FISRE method. For sake of clarity, it is chosen to describe the experimental results, ligand by ligand i.e. DOTA and DTPA for CE-ICP-MS and to compare then to the FISRE results for both ligands.

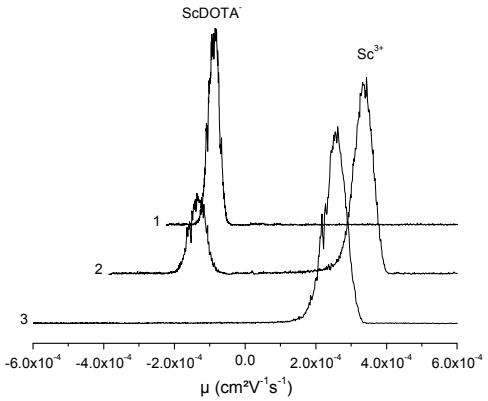


Figure 1: Electropherograms of Sc(III) at pH = 1.4, at 25°C in 0.1M of NaCl for various DOTA concentrations (1 [DOTA]=10^{-2}M; 2 [DOTA]=4.64.10^{-4}M; 3 [DOTA]=2.15.10^{-6}M)

4.1. CE-ICP-MS on Sc(III)-DOTA system

On the Sc-DOTA system, a typical electropherogram is represented in Figure 1. The electrophoretic mobility does not vary significantly but there are 2 peaks with variable areas depending on the initial concentration of DOTA.

As for low concentrations of DOTA, there was only one peak observed before the DMF one. This corresponds to cationic species, meaning thus that this peak would correspond to Sc^{3+}. When increasing the concentration of DOTA, the μ_{app} values were either positive or negative, indicated the presence of cationic species (Sc^{3+}) and/or anionic species (complex Sc(III)-DOTA) respectively. For C_{DOTA} > 1.9.10^{-6}M, the μ_{app} remains constants, indicating that the complexation is total. The overall apparent mobility was therefore be attributed to Sc(III)-DOTA complex, i.e. $[Sc\text{-}DOTA]^-$ complex (see figure 1). In addition, whichever the Sc(III)-DOTA concentration used, the mobilities do not vary. This means that the $Sc\text{-}DOTA^-$ complex is stable with regards to the electrophoretic separation.

A summary of the different protonation constants is given in Table 1, together with the *log* α corresponding to the experimental conditions used and which are taken into account for the calculation of the conditional constants of Sc(III)-complexes.

Table 1: pK_i of DOTA and DTPA at I = 0.1 M and 25 °C; calculation of corresponding α_{pH} and experimental pH used in this study.

DTPA [33]

pK_1	pK_2	pK_3	pK_4	pK_5	pK_6	pK_7
10.51 ± 0.01	8.54 ± 0.02	4.31 ± 0.02	2.55 ± 0.05	1.80 ± 0.05	1.60 ± 0.15	1.45 ± 0.15

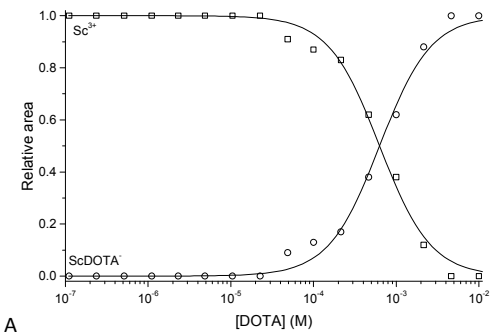
pH	1.35 ± 0.05	1.40±0.04	1.85 ± 0.05	2.11 ± 0.05	2.94 ± 0.13
Log α	21.7 ± 0.7	21.3±0.7	19.0 ± 0.6	17.8 ± 0.4	14.7 ± 0.2

DOTA[30]

pK_1	pK_2	pK_3	pK_4	pK_5
12.30 ± 0.01	9.72 ± 0.02	4.60 ± 0.02	4.10 ± 0.05	2.40 ± 0.05

pH	1.42±0.04	1.44 ± 0.07	2.32 ± 0.05
Log α	26.1±0.2	26.0 ± 0.2	22.6 ± 0.2

From Figure 2A, the inflexion is obtained for [DOTA] = (6.31 ± 0.36)10^{-4} M. Since the pH of the background electrolyte varied before and after the electrophoretic analysis, an average value of the experimental pH measured was used for the modelling (i.e. pH=1.44 ± 0.07). At this pH value, a conditional constant of $log(\alpha_{DOTA})$ = 26.0 ± 0.2 was obtained, leading to log *K* = 29.2 ± 0.2 for *I*=0.1M, as reported in Table 2. Using the Davies equation, it was possible to extrapolate this value to zero ionic strength for determining the thermodynamic constant. This value was found to be $log\ K^0$ = 31.7 ± 0.2. The *log K* = 29.2 ± 0.2 for I=0.1M is in good agreement with the one reported by Pniok et al. [30] being 30.79 ± 0.03 determined by potentiometric titration. Nonetheless, from speciation data published [30], a monoprotonated complex had to be involved into chemical speciation model, and its stability was determined from the ^{45}Sc NMR spectroscopic data (pH range 1.0-1.5), whereas in the present work only ScL specie was envisaged. Indeed, we suppose that we have a ScL specie (which is a negative specie) and not ScHL specie since the proton exchange should be fast and only an average mobility could be observed. This assumption was confirmed at ultra-trace concentrations by the means of FISRE as described in section 4.3.



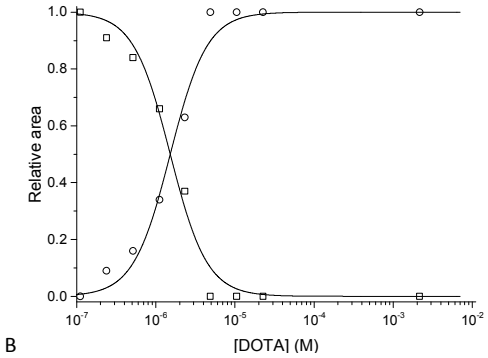


Figure 2: area ratio as a function of DOTA, in 0.1M of NaCl and T=25°C; A = pH 1.4, B = pH 2.5. [Sc^{3+}] = 5.10^{-6}M. The stability constants are determined by minimizing the function (Eq. 2) by the Levenberg-Marquardt algorithm. The mathematical models used to fit the experimental data do not take protonated, hydrolyzed and polynuclear complexes into account in the fitting procedure. These complexes have not been considered since their stabilities under the chemical conditions used here are not proven whereas the

experimental data are well fitted using only DOTA complexes in the fitting procedures.

4.2. CE-ICP-MS on Sc(III)-DTPA system

Two types of electropherograms, as shown in Figure 3, were observed: one average peak when the ligand or the metal was in excess (figure 3A); 2 peaks when the ligand and the metal were in close concentrations (figure 3B). The latter one is a particular case where the close concentrations of both species result in the consumption of the ligand (DTPA) present in the background electrolyte by the metal moving in its band of migration. In this case, two peaks are observed which are always merged since fast and continuous disequilibrium occurs. It reduces the velocity of one of the two species in one side of the migration band and increases the velocity of the other species in the other side. For the lowest concentrations of DTPA, since the mobility was positive and before DMF peak, it confirmed that it corresponded to Sc^{3+}. At the opposite, for the higher concentrations of DTPA, the negative mobility indicated that it corresponded to anionic specie either $ScDTPA^{2-}$, $ScHDTPA^{-}$ or a mix of both complexes. For the Sc-DTPA system at pcH =1.35, a single peak was observed on electropherograms, whichever DTPA concentration was used as shown in Figure 3A. Nonetheless, the mobility of this peak regularly varied with the concentration of DTPA. This meant that labile complexes were formed in solution for which fast reactions of formation/dissociation occurred in the Sc(III) band of migration. The variation of the mobility of this peak was modelled in order to calculate the complexation constant, as shown in Figure 4. Three set of independent experimental data were used to calculate the formation constant of $ScDTPA^{2-}$.

The equation used to this aim is given here under:

$$\mu = \frac{\mu_{Sc}}{1+\beta_{app}\frac{[DTPA]_{total}}{\alpha}} + \frac{\beta_{app}\frac{[DTPA]_{total}}{\alpha}\mu_{ScDTPA}}{1+\beta_{app}\frac{[DTPA]_{total}}{\alpha}} \qquad \text{Eq. 23}$$

From these data, the apparent constant could be determined and the fitting presented in Figure 4 lead to the following value: $log\ \beta_{app}{}_{pH=1.35} = 26.52 \pm 0.34$ as reported in Table 2 and Table 3. In the present work, pH is about 1.35, due to the few different pH values used; it was difficult to assign an uncertainty. We were not able to clearly determine a *log* K_H of ScHDTPA specie since it should be determined at very acidic values (i.e. pH < 1). From the log β ScL and ScHL determined by Pniok et al [30] by potentiometric titration (in the pH range from 1.5 to 12) completed by ^{45}Sc NMR spectroscopy (pH=0.8-1.3), it was possible to calculate the log β_{app} for both ligands. They were found to be log β_{app}= 27.74 and 30.94 for DTPA and DOTA respectively. The complexation constant determined in this work, in standard conditions, is given in the Table 2. Those values were in very good agreement with the one previously determined by Pniok et al. [30]. For information, this value could be calculated by extrapolating to zero ionic strength using the Davies equation, the stability constants determined by CE-ICP-MS at I=0.1 M (see Table 2).

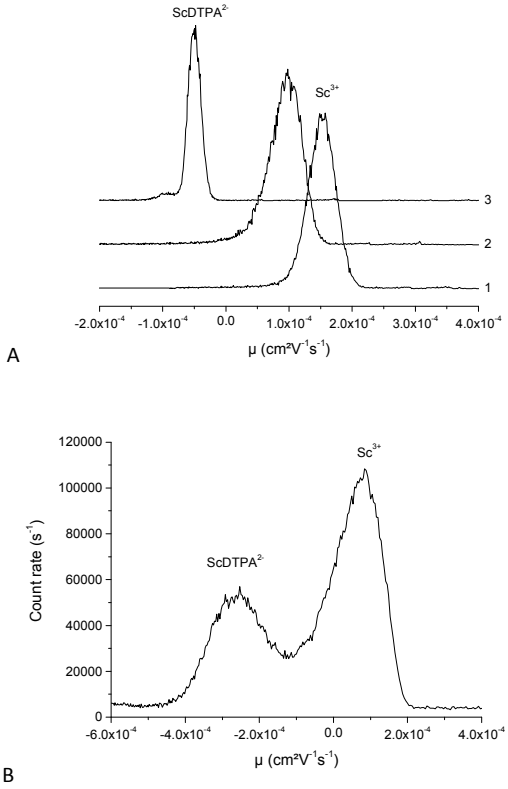


Figure 3: A Electropherograms of Sc(III) at pH = 1.3, at 25°C in 0.1M of NaCl for various DTPA concentrations (1 [DTPA]= 1.11 10^{-7}M; 2 [DTPA]=4.88.10^{-6}M; 3 [DTPA]=2.15 10^{-5} M) **B**: Typical electropherogram at pH = 3, 25°C in 0.1M NaCl for [Sc^{3+}]=5.10^{-6}M and [DTPA]=4.6 10^{-7} M, pH 3.

In that case, the complexation constant is *log* K^0 = 29.7 ± 0.7. When increasing the pH from 1.35 to 1.85, a unique peak was observed, thus only mobilities were used from this data set. The same type of treatment was performed than the one used for data acquired at pH 1.35. The fitting of the experimental data lead to a $log\ \beta_{app}{}_{pH=1.85} = 24.36 \pm 0.27$ (see Table 3).

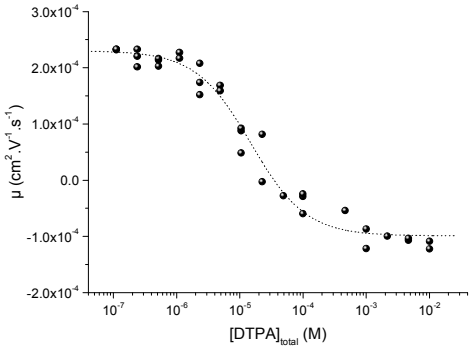


Figure 4: Electrophoretic mobility of scandium as a function of DTPA concentration. T= 25°C in 0.1M NaCl at pH=1.35.

Table 2 : logK of Sc(III)-complexes and log β_{app} at T=25°C for I=0.1M.

	CE-ICP-MS	FISRE (this work)	FISRE data at pH = 5 [12]	Potentiometric titration data [30]
DOTA				
Sc + L = [Sc(L)]	29.2 ± 0.2	29.3 ± 0.2	22.0 ± 0.5	30.79 ± 0.05
[Sc(HL)] = [Sc(L)] + H				1.36 ± 0.05
DTPA				
Sc + L = [Sc(L)]	26.52 ± 0.34	26.6 ± 0.2	22.5 ± 0.5	27.43 ± 0.05
[Sc(HL)] = [Sc(L)] + H				1.00 ± 0.05

Table 3: log K of Sc(III)-DTPA complexes at T=25°C for I=0.1M (n.a. not applicable) at various pH

pH	*Log K* at T=25°C for I=0.1M	*Log K* at T=25°C for I=0.1M
	From mobilities	From peak areas
1.35 ± 0.05	26.52 ± 0.34	n.a.
1.85 ± 0.05	24.36 ± 0.27	n.a
2.11 ± 0.05	23.44 ± 0.23	23.67 ± 0.23
2.94 ± 0.13	21.27 ± 0.51	21.03 ± 0.52

By keeping increasing the pH up to 2.11, 2 peaks appeared in the resulting electropherograms when concentrations of ligand and metal were quite similar. It was possible to calculate the conditional constant being $log\ \beta_{app_{pH=2.11}} = 23.44 \pm 0.23$ (see Table 3). The same conditional constant was calculated by using the normalized surface areas close to the equivalence point (see Figure 5).

It was calculated with the following relation: $log\beta_{app} = -log[DTPA]_{equivalence} + log\alpha$; leading thus to the following value $log\ \beta_{app_{pH=2.11}} = 23.67 \pm 0.23$ (see Table 3).

The conditional constant was calculated by using the normalized surface areas close to the equivalence point (see Figure 5) using the same equation than above. The following value $log\ \beta_{app_{pH=2.94}} = 21.03 \pm 0.52$ was obtained, whereas with the mobilities, this value was found to be $log\ \beta_{app_{pH=2.94}} = 21.27 \pm 0.51$ (see Table 3).

The different conditional constants obtained on the Sc-DTPA system are summarized in Table 3. The different results confirm that we are not dealing with a true thermodynamic constant as it depends on pH. A second form of complex was added in the fitting procedure, but the dispersion of the points and their number were too important; leading to the impossibility to extract a value. Therefore, based on the published work of Leguay et al. [33], who had studied the system An(III) /DTPA in this pH range, the values of the constants were expressed as a function of the proton concentration (i.e. pcH). By representing the log β_{app} as a function of this pcH, and by forcing the slope either at – 4 and – 3 (actual results were respectively –3.91 and –2.92) as shown in Figure 6. It is clear that the difference observed of ± 1 in the slope is due to the existence of

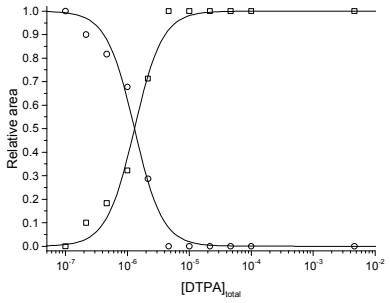


Figure 5: Normalized surface areas of Sc^{3+} and Sc-DTPA species.

another complex with a difference in the charge of 1 unit compared to the specie assumed to be present in acidic region. In other words, for pH values < 1.8 the major specie of Sc-DTPA complex is the protonated one $ScHDTPA^-$. Thus, the pK_H of the reaction $ScHDTPA^- \rightleftharpoons ScDTPA^{2-} + H^+$ is hard to be determined since in Figure 6, only 4 experimental points. pK_H is about 2.0 but it is not reasonable to assign an uncertainty. It might be reasonable to assume that the *pK* of the above reaction should range between 1.5 and 2.0.In that case, the overall apparent mobilities μ_{app} (see Eq. 2) obtained at the different pH values were not significantly different to be able to clearly determine a *log* K_H of ScHDTPA specie since it should be determined at very acidic values (i.e. pH < 1). In that case, to reach these pH values, the ionic strength would be higher than 0.5M, not allowing any extrapolation with Davies equation.

4.3. FISRE data on Sc(III)-DOTA and Sc(III)-DTPA systems

Experimental data used in the FISRE method to get stability constants for Sc(III)-DOTA and Sc(III)-DTPA complexes are presented in Figures 7A and 7B respectively. The stability constants obtained by the FISRE method are summarized in Table 2. The measurements were carried out at acidic pH; the pH conditions were determined taking into account *log β(ScL)* values as obtained from equilibrium studies (above). The stability constants obtained by the FISRE method are summarized in Table 2.

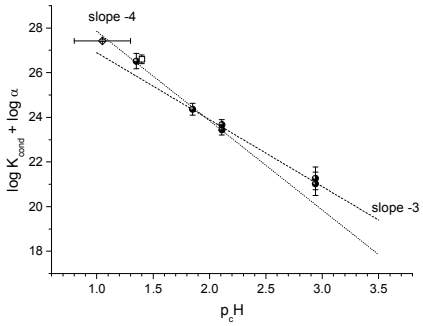


Figure 6: Variation of the conditional constant log β_{app} for the Sc-DTPA system with the concentration of proton p_cH; ● EC-CPMS; ◇ NMR ^{45}Sc [30], FISRE □.

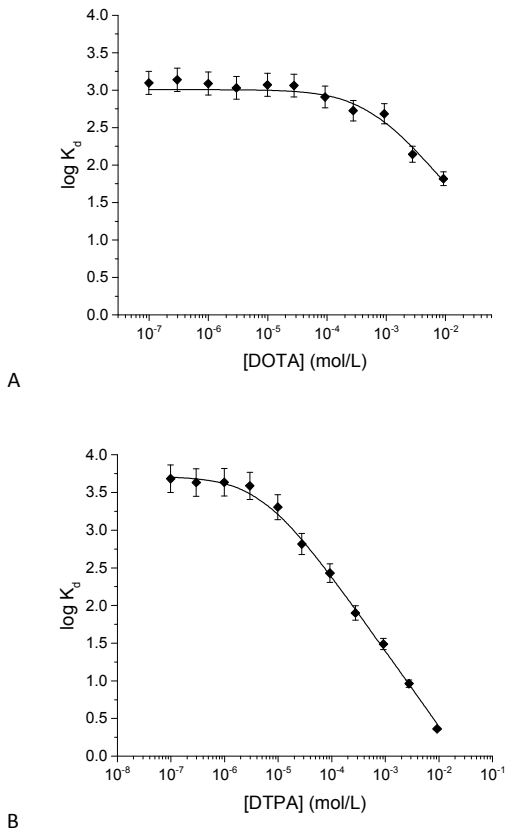


Figure 7: The Sc(III)-ligand isotherms obtained by the FISRE method: (A) DOTA; (B) DTPA. The lines correspond to the fitting as explained in SI. Experiments were performed in 0.1M NaCl solution.

The *log β(ScL)* = 29.3 ± 0.2 for DOTA and the *log β(ScL)* = 26.6 ± 0.2 for DTPA obtained by the FISRE method were in a reasonable agreement with the values obtained by potentiometry at 0.1M [30], if errors naturally accompanied utilization of trace concentrations of reactants and very high absolute values of the constants were taken into account. This value was also in quite good agreement with data published by Majkowska et al. [24] on Sc(III)-DOTA (logβ(ScL) = 27.0) determined by HPLC under higher overall metal and ligand concentrations. In all cases, the stability constants of Sc(III) were higher than 20 in log unit confirming the strong interaction between DTPA/DOTA and the trivalent scandium. Nonetheless, discrepancies of 7 orders of magnitude for the same systems between the present and previously published data [12] have been observed. This was surprising and against thermodynamic principles. A possible explanation to these discrepancies might be in a certain extent due to the possible existence of ScLH specie but that does not explain such a huge difference. More probably, since these types of ligands are not specific to a metal, if there are other metallic impurities present in the solutions, the complexation is thus affected. In that case, there is no specific way to monitor that there is saturation or a competition with the others metals present. From our previous FISRE data, the experiments were performed using a ^{46}Sc tracer of which specific activity was very low. Indeed, one of the major criteria for a radiopharmaceutical is the specific activity (SA). Specific activity—a measure of the radioactivity per unit mass of the compound—is an indicator of potency; the higher the specific activity of a radionuclide, the higher both the percentage of radioactive atoms and the deliverable dose. Since the ligands considered in the present work are not specific to scandium only, they can complex every metal in solution so the main difference observed in the ligand concentration is due to the concentration of the metal in solution to get suitable radiolabelling yield. Specific activity may or may not be important depending on the number of sites available for targeting. It is defined as:

$$SA = \frac{A}{[Sc(III)]} \qquad \textit{Eq. 24}$$

Where A is the activity in Bq and [Sc(III)] is the total scandium concentration in mol. But the operational specific activity is:

$$SA_{op} = \frac{A}{\sum[metals]} \qquad \textit{Eq. 25}$$

With [metals] corresponding to the sum of all metallic impurities contained in the solution, given in mol.

Thus, the discrepancies observed between our previous set of data [12] and the one form the present work are most probably linked to the specific activity. But above all these considerations, the discrepancies observed could rather be due to inappropriate pH conditions (ie pH 5) used for the determination of log β(ScL) by FISRE [12]. Indeed, at pH= 5 the complexation of Sc(III) by DOTA was almost total, and then no equilibrium data could be determined precisely. Nonetheless, as the FISRE method employs only trace concentrations of reactants, its main advantage was that it can be used also for "problematic" metal ions those stability constants can be hardly determined by common methods as potentiometry. The potential metal ions of radiopharmaceutical interest include e.g. easily hydrolyzing metal ions as Zr(IV), Bi(III), Ac(III) or Th(IV). Data presented in this paper clearly showed that the FISRE method was more easy to carry out, faster and operationally cheaper than the "standard" methods (stability constant of the $[Sc(DOTA)]^-$ complex could not be determined by the conventional methods here) and gave results which could be used for evaluation of complexation ability of new ligands toward metal ions to be utilized as radiopharmaceuticals.

4.4. Discussion on the methodology of determination of thermodynamic parameters

As previously highlighted by Anderegg et al. [35], the solubility of complexones such as DTPA and DOTA attains a minimum between pH 1 and 4. This causes considerable accuracy problems in the measurement of protonation constants (*log K_i*) of the neutral species H_iL and the ion $H_{i+1}L^+$. The use of an incorrect or incomplete set of *log K_i* values may result in appreciable errors in calculated stability constants for highly stable complexes, for which measurements at low pH are required. A very common error

involves the neglect of positively charged ligand species that exist between pH 0 and 2; this is pertinent to spectrophotometric, electromigration, and other methodologies for the measurement of stability constants. Nonetheless, for DTPA and DOTA, discrepancies in *log K*(L + H) values and *log K*(HL + H) were shown to be due to the binding of these anions to alkali metal ions of the background electrolytes used (i.e. in the present case Na^+). These alkali cations can efficiently compete with protons for nitrogen donor atoms of such complexones and their interaction is rather strong. Especially, in the case of DOTA, it forms stable complexes with Na^+ that lead to anormalously low values for *K*(L + H) and *K*(HL + H). Another problem with DOTA measurements is the high value for the first protonation constant, *K*(L + H). In such cases, log K is difficult to determine by the usual potentiometric methods. NMR titration is the preferred technique. This has been the case in the determination of protonation constants of DOTA by Pniok et al. [30]. Thus, for *log* K_{ML} calculations, especially in the case of DOTA and DTPA, ligand protonation constants obtained in solutions should take into account Na^+ complexation for further correction. As already mentioned by Anderegg el al. [35], the determination of stability constants for metal complexes of DOTA is highly dependent on the values used for *K*(L + H) and *K*(HL + H). One of the reasons for the spread of values found in the literature for stability constants of complexes with this ligand is the variety of *K*(L + H) values used by different authors. Those working with supporting Na^+ electrolytes always report lower values of K_{ML}. Then the stability constant (expressed in log) requires corrections by a factor of $log(1 + K_{NaDOTA}[\mathrm{Na}])$. In the present work with NaCl = 0.1 M, *log* K_{NaDOTA} = 4.2 [36]; thus the correction is + 3.2 at this ionic strength, which is very important. If one extrapolates that to zero ionic strength, this correction is even higher (> 6). The determination of stability constant requires either "neutral" (indifferent) cations for the system (i.e. tetramethylammonium or ammonium ions or for any other supporting electrolytes, corrections of the interaction of the corresponding cation with the ligands. Potentiometry is a suitable technique for the determination of interaction constant in these conditions. To illustrate this purpose, Pniok et al. [30] have determined the stability constant of scandium with DOTA and DTPA by potentiometry and NMR, as highlighted by Anderegg et al. [35]. Potentiometry requires weightable amounts of both compounds, metal and ligand; and seems to be an "anywhere applicable" technique. One limitation is that if the interaction constant between an element of interest and a ligand is very high, potentiometry would allow only the determination of a limit value of that constant. Over that limit value of the constant, if the ligand is fully deprotonated due to the interaction with the element of interest, it would be impossible to discriminate the ligand from the complex. From data obtained in this work, CE-ICP-MS is a method of choice since it allows a direct speciation whereas potentiometry or FISRE are indirect methods in which different species are not directly determined. Capillary electrophoresis (CE) is a separation technique with high resolution and does not change the speciation of the system studied. ICP-MS works at the scale of trace, for which the limit of solubility of the ligands is rarely reached. One of the limitations of CE-ICP-MS might be the cost of the device since the ICP-MS modality could be costly, and thus not affordable to any lab. If one wants to establish thermodynamic data, the second limitation is also that ICP-MS may be not directly applicable to any element of the periodic table. Indeed, if the element of interest could be analyzed by that technique, one should be aware of the possible interferences and the detection limits for that element. Finally, CE-ICP-MS is a suitable tool for determining complexation constant only if there is a significant variation of the complex charges. To circumvent this potential issue, Free Ion Selective Radiotracer Extraction (FISRE), could be an alternative method applicable at trace concentrations like for CE-ICP-MS. Both methods are extendable to the use of radionuclides combining radiosafety aspects due to the low amounts necessary. One limitation of this technique is that if ligands are not specific to a metal (or any other element) such in the present work, the ligand may have the same range of interaction with others metallic competitors than for the element of interest. In that case, it would be impossible to determine an interaction constant. In addition to that, from the experimental point of view, it is necessary to find out the suitable conditions that allow a strong sorption onto the resin, leading to an impoverishment of the aqueous phase; but not too strong in order to desorb the metal (or the element of interest) by the ligand addition in the system.

Conclusions

To obtain a confident starting point for a future research on ligands suitable for pharmaceutical applications of scandium radioisotopes, we investigated scandium(III) complexes of two "parent" ligands, DTPA and DOTA. Literature [12] showed that DOTA and DTPA were the two most favourable ligands for scandium since they exhibited the two highest stability constants with regards to other polyaminopolycarboxylic ligands. Stability constants of scandium(III) complexes of both ligands are very high but depending on the method used for their determination, potentiometric titration or ion exchange resin, main discrepancies have been found in literature and from our previous work, which is from a thermodynamic point of view, totally inconsistent.

Thus, this work has examined the complexation of scandium with DTPA and DOTA by the coupling of capillary electrophoresis (CE) coupled to an ICP - MS. We observed that the use of CE ICP - MS was effective for the determination of complexation of the scandium complex constants - DOTA or DTPA. The constants obtained by this method at trace concentrations, were in agreement with those obtained by potentiometric method. As the complexes are fully formed even below pH 2, protonation constants of both DTPA and DOTA had to be re-investigated and the lowest (acidic) constants important in this low-pH region were determined. The presence of protonated and deprotonated complexes was also suggested. The stability constants obtained by the FISRE method were in a reasonable agreement with the values obtained by potentiometry at 0.1M, if stability constants for

the monoprotonated complex *logβ*(ScHL) were involved into the FISRE data fitting and if errors naturally accompanied utilization of trace concentrations of reactants were taken into account. The information presented in this paper may be used as standard data for investigations of aqueous chemistry of scandium(III) complexes with polydentate ligands, by bringing new thermodynamic data and by completing the panel of available metals in medicine. Our results support the argument that DOTA and DTPA ligands are the two most favorable chelated to be coordinated to scandium, as already published [12]. In the presence of a challenging protein such as transferrin, the equilibrium was not reversible on the time scale of couple hours for DTPA and DOTA whereas a fast transfer of scandium(III) to transferrin occurred for the Sc-TETA complex for instance from the first contact. Those two ligands were assessed as far as radiolabelling with ^{44}Sc was concerned exhibiting ratio > 90% and > 80 % for Sc-DOTA and Sc-DTPA , respectively, for a Sc:L molar ratio of 1:1. The study of the stability in the presence of hydroxyapatite (a bone mimic) and rat serum, indicated that Sc-DOTA was the most suitable in the perspective of medical applications[6].

Finally, this work has reviewed and experienced several types of methods for the determination of thermodynamical data. If one wants to determine thermodynamic data, most of the time the device present in the lab are used. Nonetheless, for the assessment of robust thermodynamic data, crossed techniques at different scales must be used, each one having their limitations, and suitable conditional parameters are crucial. The key point is that any method allowing the determination of equilibrium should be set with caution with regards to the physico-chemical conditions for any bi-phasic system (pH, resin or organic phase).

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