

Revisiting Lead(II)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic Acid Coordination Chemistry in Aqueous Solutions: Evidence of an Underestimated Thermodynamic Stability

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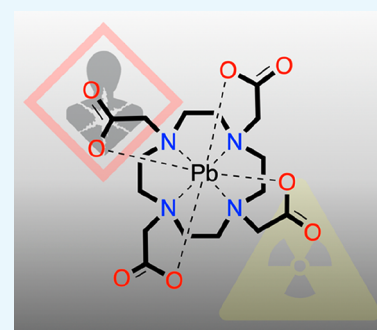


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ABSTRACT: The complexes formed between Pb^{2+} and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) were reinvestigated in aqueous solutions using a combination of pH potentiometry, UV–vis spectroscopy, and NMR spectroscopy. The thermodynamic data were supported by kinetics assays. Differently protonated complexes, *i.e.*, $[\text{PbH}_3\text{L}]^+$, $[\text{PbH}_2\text{L}]$, $[\text{PbHL}]^-$, and $[\text{PbL}]^{2-}$, were detected, and the corresponding stability constants ($\log\beta$) at $T = 298$ K and $I = 0.1$ M NaCl were 33.1 ± 0.2 , 32.00 ± 0.06 , 29.28 ± 0.06 , and 25.3 ± 0.1 , respectively. Results differed significantly from those previously reported by Chaves *et al.* (*Talanta* 1992, 39, 249) and Pippin *et al.* (*Inorg. Chim. Acta* 1995, 239, 43) in both the speciation and the overall complex stability; the latter in particular was found to be remarkably higher. The work disclosed herein provides revised data on the Pb^{2+} -DOTA complexes, which should be used as a new stability benchmark during the development of lead chelators.



INTRODUCTION

The lead radioisotopes, ^{203}Pb and ^{212}Pb , are attracting growing interest in targeted cancer radiotherapy and radioimaging as they form an ideal theranostic pair.^{1–3} Lead radionuclides can be safely driven to the biological target if they are chelated by an appropriate ligand (conjugated to a tumor-seeking vector), forming a highly thermodynamically stable and kinetically inert complex *in vivo* with Pb^{2+} .^{4–8}

To date, the chelation of lead radioisotopes has been mainly explored with two ligands, the widely used 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and its tetracetamide derivative 1,4,7,10-tetrakis-(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane (DOTAM or TCMC) (Figure 1). However, there has been a renewed and increased search for alternative ligands that, better than DOTA and DOTAM, satisfy the stringent demands placed on a metal chelator for use with lead radioisotopes in nuclear medicine applications.^{1,9–16} Highly specific Pb^{2+} chelators are also extremely important for lead poisoning prevention and

treatment and the remediation of contaminated liquids or environments because of the elevated human toxicity of this heavy metal.¹⁷

When a novel Pb^{2+} chelator is proposed, the first gauge of its performance is given by the thermodynamic properties, *i.e.*, metal–ligand speciation and complex stability constants ($\log\beta$), which can be compared with those available in the literature for state-of-the-art DOTA or DOTAM. Data for Pb^{2+} -DOTA were given in two rather old manuscripts. In 1992, Chaves *et al.* detected two 1:1 and two 2:1 metal-to-ligand complexes and reported the corresponding $\log\beta$ values obtained by potentiometric titrations (Table 1).¹⁸ In 1995, Pippin *et al.* restudied the Pb^{2+} -DOTA complexes *via* competitive titrations with ethylenediaminetetraacetic acid (EDTA) through UV–vis spectroscopy and detected only two 1:1 complexes (Table 1).² The number, stoichiometry, and stability constants of Pb^{2+} -DOTA complexes should be correctly established due to their importance in the above-mentioned research fields. Furthermore, according to our evidence, the stability constant values were underestimated in both studies. Herein, we report a detailed reappraisal of the thermodynamic properties of the Pb^{2+} -DOTA complexes in aqueous solutions. A ionic strength of 0.1 M NaCl was chosen

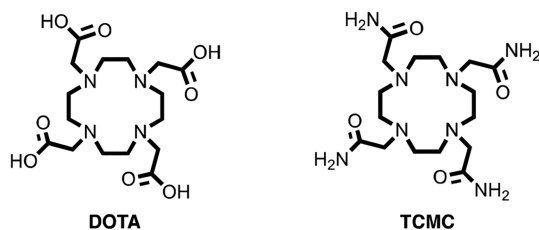


Figure 1. Structures of DOTA and TCMC.

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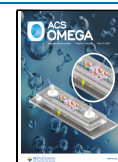


Table 1. Acidity Constants of DOTA (pK_a) and Stability Constants ($\log\beta$) of Pb^{2+} -DOTA Complexes Obtained in the Present Work ($I = 0.1$ M NaCl)^a

reaction ^b	pK_a ^b		
$H_3L \rightleftharpoons H_3L^- + H^+$	4.63 ^c	4.09 ^d	4.13 ^e
$H_3L^- \rightleftharpoons H_2L^{2-} + H^+$	4.86 ^c	4.559 ^d	4.67 ^e
$H_2L^{2-} \rightleftharpoons HL^{3-} + H^+$	9.18 ^c	9.76 ^d	9.70 ^e
$HL^{3-} \rightleftharpoons L^{4-} + H^+$	11.43 ^c	12.09 ^d	12.00 ^e
reaction ^b	$\log\beta$		
	this work ^f	Chaves <i>et al.</i> ^d	Pippin <i>et al.</i> ^g
$Pb^{2+} + L^{4-} \rightleftharpoons [PbL]^{2-}$	25.3 ± 0.1 ^h 24.9 ± 0.1 ⁱ	22.69	24.3
$Pb^{2+} + H^+ + L^{4-} \rightleftharpoons [PbHL]^-$	29.28 ± 0.06 ^j 28.8 ± 0.1 ⁱ	26.55	27.6
$Pb^{2+} + 2H^+ + L^{4-} \rightleftharpoons [PbH_2L]$	32.0 ± 0.06 ^j 31.7 ± 0.1 ⁱ		
$Pb^{2+} + 3H^+ + L^{4-} \rightleftharpoons [PbH_3L]^+$	33.1 ± 0.2 ⁱ 32.8 ± 0.1 ⁱ		
$2Pb^{2+} + L^{4-} \rightleftharpoons [Pb_2L]$		25.99	
$2Pb^{2+} + H^+ + L^{4-} \rightleftharpoons [Pb_2HL]^+$		29.66	
pPb^{2+k}	20	16	18

^aLiterature data are reported for comparison. Unless otherwise stated, the reported data are referred to $T = 298$ K. ^bL denotes DOTA in its fully deprotonated form. ^cFrom ref 20, $I = 0.15$ M NaCl. ^dFrom ref 18, $I = 0.1$ M Me_4NNO_3 . The method was potentiometry. ^eFrom ref 2, $I = 0.1$ M NMe_4Cl . ^fThe reported uncertainty was obtained *via* the fitting procedure and represents one standard deviation unit. ^gFrom ref 2, $I = 0.1$ M $NaClO_4$. The method was competitive UV–vis spectrophotometric titrations with EDTA. ^hThe method was UV–vis spectrophotometric titrations. ⁱ $T = 338$ K. The method was NMR. ^jThe method was potentiometry $k_pPb^{2+} = -\log[Pb^{2+}]$, where $[Pb^{2+}]$ was calculated at $C_{Pb^{2+}} = 10^{-6}$ M and $C_{DOTA} = 10^{-5}$ M at pH 7.4.

in this study to more directly relate our results to a blood plasma environment.

RESULTS AND DISCUSSION

Pb^{2+} -DOTA Formation Kinetics. The first and obvious requirement to allow rigorous equilibrium data to be obtained in a complex-formation study is that the considered reactions must be at equilibrium when data is collected. This requirement was verified by a preliminary kinetics study in which the time necessary to reach the equilibrium state was qualitatively assessed by mixing Pb^{2+} and DOTA at various pH and concentrations. This study was performed using UV–vis spectroscopy. Figures S1 and S2 show the kinetic data obtained at metal and ligand concentrations of 10^{-4} and 10^{-3} M, respectively, which represent the two typical concentration sets employed in the subsequent thermodynamic investigation.

The absorption centered at 258 nm, characteristic of the complexation event, is in agreement with that previously reported in the literature and is assigned to the $6s^2 \rightarrow 6sp$ transition of Pb^{2+} .^{2,19} The pH increase in the aqueous medium progressively accelerates the complexation reaction; when the concentration of the two reactants was set to 10^{-4} M, the equilibrium was reached in ~ 3 h at pH 2.0 and in ~ 20 min at pH 3.0, while the reaction took place during the mixing time at pH 7.4 (Figure S1). On the other hand, at a concentration of 10^{-3} M, the complexation reaction reached equilibrium in ~ 10 min at pH 2 and became instantaneous at pH 4.5 (Figure S2). The lower reaction rate at the more acidic pH is ascribed to

the increase of the electrostatic repulsion between the metal ion and the protons on the donor sites, which become progressively more protonated (secondary amines of the macrocycle and acetate groups of the side arms).^{20–22}

Pb^{2+} -DOTA Solution Thermodynamics. The stability constants ($\log\beta$) of Pb^{2+} -DOTA at $T = 298$ K and $I = 0.1$ M NaCl, summarized in Table 1, were determined using a combination of pH potentiometry, UV–vis spectroscopy, and 1H NMR spectroscopy. The corresponding distribution diagram is shown in Figure 2, and some typical fitting lines obtained for the potentiometric titrations are shown in Figure S3.

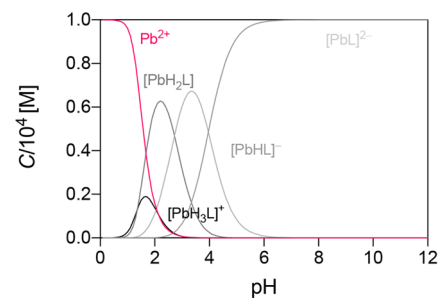


Figure 2. Distribution diagram of Pb^{2+} -DOTA at $C_{Pb^{2+}} = C_{DOTA} = 1 \times 10^{-4}$ M, $I = 0.1$ M NaCl, and $T = 298$ K.

At a concentration of 10^{-3} M, the reaction between Pb^{2+} and DOTA is relatively rapid (*vide supra*), making the potentiometric method applicable and allowing the speciation model, which involves the presence of differently protonated 1:1 metal–ligand complexes, *i.e.*, $[PbH_2L]$, $[PbHL]^-$, and $[PbL]^{2-}$, to be determined (Table 1). The pK_a values of $[PbH_2L]$ and of $[PbHL]^-$ could be determined by potentiometry. However, only a lower limit for the $\log\beta$ values can be estimated using this technique due to the high stability of the resulting complexes, which causes the complexation to start at pH levels much lower than 2. The $\log\beta$ values were thus determined by UV–vis spectroscopy.

The UV–vis spectra obtained at different pH values from in-batch pH-dependent spectrophotometric titrations are shown in Figure 3. The progressive increase of the absorption band maximum in the investigated pH range (Figure S4) is attributed to the formation of the complexes. The low spectral resolution did not allow evidence of the formation of different protonated species; thus, pK_a values obtained from pH-potentiometry and NMR (*vide infra*) were inserted as constant

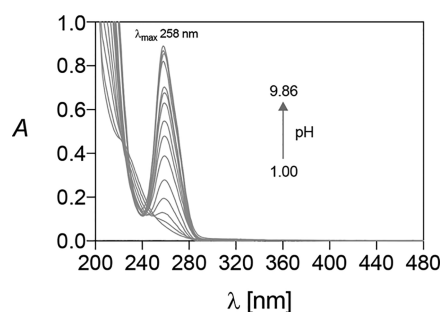


Figure 3. Representative UV–vis spectra of Pb^{2+} -DOTA solutions at equilibrium ($T = 298$ K, $I = 0.1$ M NaCl, and $C_{Pb^{2+}} = C_{DOTA} = 1 \times 10^{-4}$ M) at different pH values.

parameters in the fitting procedure, allowing the determination of $\log\beta$ value of $[\text{PbH}_3\text{L}]^+$ from which all other formation constants given in Table 1 were computed.

The overall complex stability was evaluated by computing pPb^{2+} ($= -\log[\text{Pb}^{2+}]$); a value of 20 was obtained (at pH 7.4, $C_{\text{Pb}^{2+}} = 10^{-5}$ M, and $C_{\text{DOTA}} = 10^{-6}$ M), which was remarkably higher than the values determined using the data of Pippin *et al.*² ($\text{pPb}^{2+} = 18$) and Chaves *et al.*¹⁸ ($\text{pPb}^{2+} = 16$). According to the speciation data of Pippin *et al.* and Chaves *et al.*, Pb^{2+} and DOTA should not react significantly at pH ~ 2 , but our data show that a complex forms in this condition (e.g., see Figures S1 and S2). To further confirm that the complex is stable at acidic pH, thus proving the high complex stability determined in this work, the acid-mediated decomplexation reaction of the Pb^{2+} -DOTA complexes was evaluated by UV-vis spectroscopy. These assays also allowed us to obtain the dissociation rate constants ($k_{\text{obs}}^{\text{d}}$) and the corresponding half-lives ($t_{1/2}$) at different HCl concentrations, which are compiled in Table S1. Figure S5 shows the observed variation of the electronic spectra over time at the different pH levels, while representative linear regressions are reported in Figure S6. The Pb^{2+} -DOTA complexes only partially decomplex at pH 2.0, while there is progressively faster and quantitative decomplexation at lower pH, which is in agreement with the thermodynamic data (Figure 2). Starting from a 10^{-4} M complex concentration, the time necessary to reach the equilibrium conditions was ~ 6 h at pH 2.0, ~ 3 h at pH 1.0, and ~ 7 min at pH 0.0. During the decomplexation at pH 0.0, the time-dependent disappearance of the peak representative of the Pb^{2+} -DOTA complex at 258 nm was accompanied by the appearance of a peak characteristic of the formation of the $[\text{PbCl}_x]^{2-x}$ complexes. This was demonstrated by the UV-vis spectra of Pb^{2+} -DOTA solutions recorded in highly acidic pH environments (pH < 1 , Figure S7). The maximum absorption at 225 nm was observed at pH 0.8, which was ascribed to the free water ion $\text{Pb}^{2+}_{(\text{aq})}$ ($\lambda_{\text{max, Pb}^{2+}(\text{aq})} = 209$ nm) undergoing a progressive bathochromic shift up to pH 0.0. At pH 0.0, the maximum absorption is at 245 nm due to lead-chloro complexes $[\text{PbCl}_x]^{2-x}$ ($\lambda_{\text{max, [PbCl}_x]^{2-x}} = 245$ nm).

Spectrophotometric titrations were also performed at different metal-to-ligand ratios and different starting ligand concentrations to evaluate the stoichiometric ratio of the metal-ligand complexes. A sharp inflection point was always observed at an equimolar metal-to-ligand ratio (Figure S8). This result confirms the exclusive 1:1 Pb^{2+} -to-DOTA binding stoichiometry, which is in agreement with the speciation obtained by Pippin *et al.*² but in disagreement with that given by Chaves *et al.*¹⁸

The speciation data obtained by potentiometry and UV-vis spectroscopy were further supported by performing a ^1H NMR investigation, which also allowed us to gain insight into the aqueous solution structure of the Pb^{2+} -DOTA complexes. NMR was also employed because, to the best of our knowledge, only a very brief note about the NMR properties of Pb^{2+} -DOTA complexes has been provided so far.¹⁹

The ^1H NMR spectra of free DOTA and Pb^{2+} -DOTA solutions at 298 K are reported in Figures S9 and 4, respectively. The spectra of free DOTA display two signals related to the nitrogen-bound protons of the macrocyclic ring and the methylenic protons of the acetate arms. The addition of Pb^{2+} causes important variations in the chemical shifts and coupling patterns.

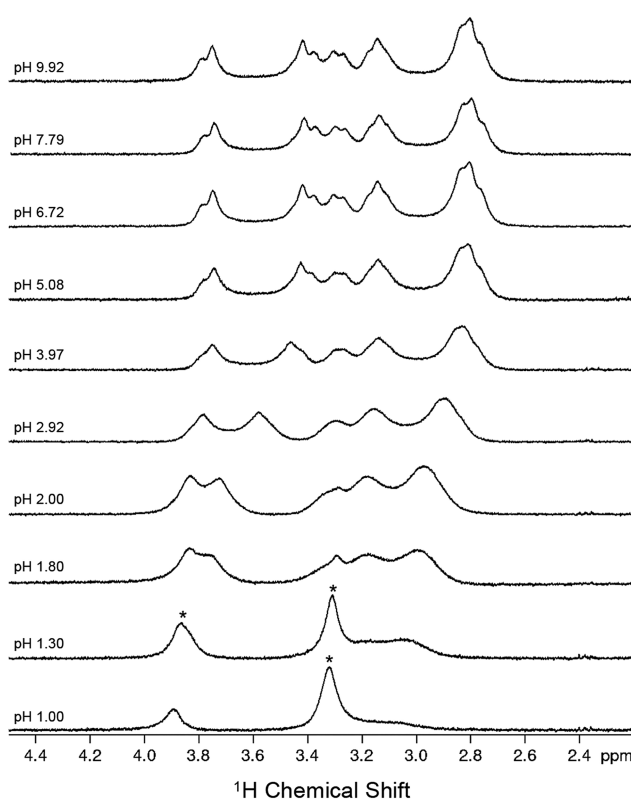


Figure 4. ^1H NMR spectra of Pb^{2+} -DOTA solutions (400 MHz, $T = 298$ K, $\text{H}_2\text{O}/10\% \text{D}_2\text{O}$, and $C_{\text{Pb}^{2+}} = C_{\text{DOTA}} = 1 \times 10^{-3}$ M) at different pH values. The signals related to the free ligand are marked with an asterisk.

These changes were first used to support the stoichiometric results from potentiometry and UV-vis spectroscopy, *i.e.*, the exclusive formation of 1:1 metal-to-ligand complexes. NMR titrations at constant pH and different metal-to-ligand ratios undoubtedly indicate the 1:1 stoichiometry because the spectra display significant changes upon the addition of Pb^{2+} when the metal-to-ligand ratio was below 1:1, whereas no further changes are observed upon the addition of Pb^{2+} equivalents larger than one (Figure S10).

Second, the spectra indicate that the Pb^{2+} -DOTA complexes coexist with the unbound (free) ligand only at very acidic pH levels (Figure 4), which is in agreement with the pH potentiometric and UV-vis data. When the pH increases, the peaks related to free DOTA gradually disappear while those attributed to the metal complexes become progressively more intense. A broad multiplet spanning between 2.85 and 3.24 ppm is noticeable at pH > 1 . This resonance is defined progressively with the decrease of the proton content, splitting into three partially overlapped multiplets at 2.80, 3.14, and 3.29 ppm at pH > 2.92 ; with the aid of ^1H - ^1H TOCSY experiment, these multiplets were attributed to the CH_2 protons of the polyazamacrocyclic scaffold (Figure S11). At the same time, the singlet attributed to the acetate protons of the free ligand (~ 3.91 ppm at pH 1) is gradually replaced by two broad resonances, which experience an increased chemical shift separation ($\Delta\delta = 0.1$ ppm at pH 2.0 and $\Delta\delta = 0.4$ ppm at pH 9.92) and end up appearing as two poorly resolved but well-separated multiplets at pH > 2.92 .

The presence of many broad signals combined with the absence of ^1H - ^{207}Pb satellites indicates dynamic coordination

between the ligand and the metal at 298 K. On the contrary, the exchange with the free ligand does not take place or occurs in a time scale greater than that of the NMR experiment, since the signals related to the free ligand are always recognizable.

At pH > 5, the spectra are unchanged, thus indicating that the same complex exists in solution. According to potentiometry, this species is $[\text{PbL}]^{2-}$. In agreement with the potentiometric data (*vide supra*), the recognizable peaks that shift toward higher fields and are detected in the range $1.0 \leq \text{pH} \leq 5.08$ can be ascribed to the copresence of the differently protonated complexes (Figure 2). However, the presence of the broad multiplets complicates both the peak selection and the relative area calculation, thus preventing the determination of unique pK_a values for the protonated species. Variable-temperature ^1H NMR spectra were thus collected to circumvent this problem and to investigate the intramolecular dynamic exchange processes that occur in the Pb^{2+} -DOTA complexes.

Figure S12 presents the ^1H NMR spectra of Pb^{2+} -DOTA solutions at several temperatures (from 278 to 338 K) and constant pH. All signals become broader as the temperature increases from 298 to 318 K (coalescence temperature), but a further increase in temperature to 338 K leads to less complicated and more resolved spectra when compared to those recorded at 298 K, likely thanks to the faster intramolecular exchange kinetics. Spectra at 338 K were thus collected as a function of the pH, and they are shown in Figure 5.

All spectra at pH > 4 are identical to each other, thus indicating the presence of only one complex that, according to the potentiometric data at 298 K, should be $[\text{PbL}]^{2-}$. The two very broad singlets at 2.97 and 3.56 ppm are associated with the NCH_2 and the acetate protons, respectively. These spectral features suggest that $[\text{PbL}]^{2-}$ is a highly symmetric octa-coordinated complex and point to a fast interconversion between isomers. In reality, the high symmetry of $[\text{PbL}]^{2-}$ was confirmed in the solid-state by X-ray spectroscopy.²³

Upon lowering the pH, the NCH_2 signals split into two quasi-symmetrical broad multiplets, whereas the acetate signals remained a singlet structure. Nonetheless, all these signals shift remarkably with the decrease in pH (Figure S13). Data fitting allowed us to determine the pK_a values for $[\text{PbH}_2\text{L}]$ and $[\text{PbHL}]^-$ that were associated with the different protonation steps (Table 1). These values are in good agreement with those obtained by pH potentiometry at 298 K, pointing out that the protonation processes are only slightly affected by temperature. The very acidic pH ($\ll 2$) investigated by NMR spectroscopy allowed to recognize the presence of an additional species with a pK_a of 1.1, which was assigned to $[\text{PbH}_3\text{L}]^+$. Despite the fact that this species was detected at 338 K, it can be assumed that $[\text{PbH}_3\text{L}]^+$ exists also at 298 K. Due to the small temperature dependence of the other pK_a values highlighted above, the pK_a of $[\text{PbH}_3\text{L}]^+$ can also be set to roughly 1.1 at 298 K.

When the samples are cooled to 278 K, all the signals become sharp (Figure S14) and their multiplicity is recognizable, thus suggesting that these complexes are present only as one conformer. At pH > 4, where $[\text{PbL}]^{2+}$ should exist, the two doublets at 3.78 and 3.40 ppm (area of 4, $^2J = 18$ Hz) were attributed to the two CH_2 protons of the acetate side arms, while the two triplets at 3.33 and 3.15 ppm (area 4, $^2J = 15$ Hz) and the two partially overlapped doublets at 2.80 ppm (area 4, $^2J = 16$ Hz) were assigned to the NCH_2 protons of the

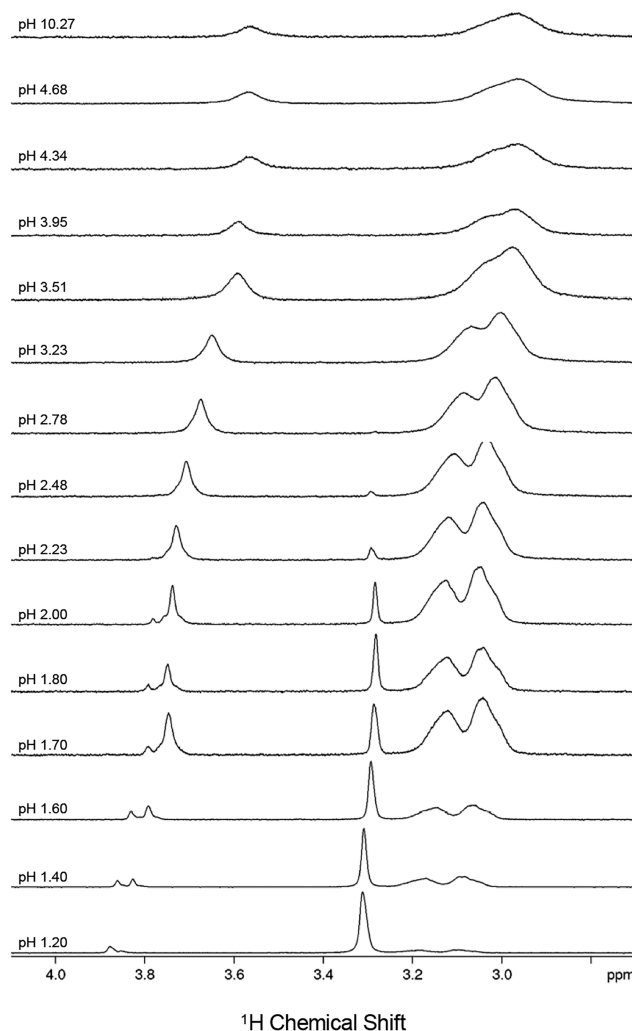


Figure 5. ^1H NMR spectra of Pb^{2+} -DOTA solutions (400 MHz, $T = 338$ K, $\text{H}_2\text{O}/10\% \text{D}_2\text{O}$, and $C_{\text{Pb}^{2+}} = C_{\text{DOTA}} = 1 \times 10^{-3}$ M) at different pH values.

ring. This spectrum is fairly similar to those reported for the $[\text{PbL}]^{2+}$ complex formed by DOTAM¹⁷ and seems consistent with a Pb^{2+} complex in which all the CH_2 protons became non-magnetically equivalent after the metal chelation, differentiating both sides of the cyclen ring. The intramolecular dynamic exchange processes observed for the $[\text{PbL}]^{2-}$ complex of DOTA, which are responsible for the line broadening observed at room temperature, are likely related to the interconversion processes that can occur *via* pendant arm rotation and macrocyclic chelate ring inversion. The estimated average activation free energies for the dynamic processes ($\Delta G^\ddagger \approx 62$ – 67 kJ/mol) are very close to those determined for DOTAM (62–64 kJ/mol).^{17,24} The NMR spectra at 278 K showed a pH-dependent variation of the chemical shift similar to that observed at 338 K (Figure S15), indicating that several protonation states are also present at this low temperature. However, the data could not be fitted and no pK_a values were here determined.

EXPERIMENTAL SECTION

Materials. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was obtained from Chematech. Lead

chloride (PbCl_2 , 99.999%) was purchased from Sigma-Aldrich. All other chemicals were purchased from commercial suppliers (Sigma-Aldrich, Fluka, and VWR Chemicals) and were used as received without further purification. All solutions were prepared in ultrapure water (18.2 M Ω -cm, Purelab Chorus system, Veolia).

Pb²⁺-DOTA Formation Kinetics. The Pb²⁺-DOTA formation kinetics was assessed through UV–vis spectroscopy using a Cary 60 UV–vis spectrophotometer (Agilent). Equimolar amounts of metal ion and ligand were mixed in buffered aqueous solutions at pH 2.0 (1.0×10^{-2} M HCl), 3.0 (1.0×10^{-3} M HCl), 4.5 (acetic acid/acetate), and 7.4 (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid - HEPES) at room temperature. The electronic spectra were collected immediately after mixing and at different time points in the range from 200 to 800 nm using a quartz spectrophotometric cell with a 1 cm path length. Different concentrations ranging from 1×10^{-4} to 1×10^{-3} M were employed.

The complexation reaction was monitored directly over time using the increase of the absorption peak characteristic of Pb²⁺-DOTA complex formation.

Pb²⁺-DOTA Solution Thermodynamics. Potentiometry. The experimental procedures and details of the potentiometric apparatus closely followed those in our reported studies.^{4,5,25} DOTA and lead stock solutions were prepared by the direct dissolution of a weighed portion of ligand and PbCl₂ in water at a 2×10^{-3} M concentration. Pb²⁺ solutions were standardized using complexometric titrations with ethylenediaminetetraacetic acid (EDTA), and xylenole orange was used as an indicator. The ionic strength was fixed to 0.1 M using sodium chloride (NaCl) as a background electrolyte. Each experiment was performed independently at least three times.

pH-Dependent and Variable-Temperature NMR. NMR spectra of free DOTA and its Pb²⁺ complexes at different pH levels were collected using a 400 MHz Bruker Avance III HD spectrometer or a 600 MHz Bruker DMX 600 spectrometer. The temperature limits investigated were set between 278 and 338 K. No temperature was considered out of this range to avoid damaging the probe due to the solvent either freezing and breaking the NMR tube or boiling and leaking. Proton chemical shifts (δ) are reported in parts per million (ppm) and are referred to 3-(trimethylsilyl)propionic acid sodium salt (Sigma-Aldrich, 99%). All the solutions were prepared in H₂O/10% D₂O (Sigma-Aldrich, 99.9% D) at a concentration of $\sim 10^{-3}$ M. The pH was adjusted with small additions of HCl or NaOH and measured with the same setup used for the potentiometric measurements. The water signal was suppressed using an excitation-sculpting pulse scheme.²⁶ All data were collected and processed with Topspin 3.5 using standard Bruker processing parameters with Topspin 4.1.1 software.

pH-Dependent UV–vis Spectrophotometric Titrations. UV–vis titrations were carried out using the out-of-cell methods in the pH range 0–12 at 298 K. Stock solutions of DOTA and PbCl₂ were mixed in independent vials to obtain a 1:1 metal-to-ligand molar ratio (final concentration of $\sim 10^{-4}$ M), and different amounts of HCl and NaOH were added to adjust the pH. The pH was measured with a Mettler Toledo pH meter equipped with a glass electrode calibrated daily with commercial buffer solutions (pH 4.0 and pH 7.0), whereas in very acidic solutions (pH \ll 2) it was computed from the HCl concentration (pH = $-\log C_{\text{HCl}}$). The absorption spectra were recorded using the same apparatus described above for the

kinetic measurements. The equilibrium was considered to be reached when no variations of the UV–vis spectra or pH were detected.

Titrations at Different Metal-to-Ligand Molar Ratios. Different aliquots of the Pb²⁺ stock solution were added to the ligand solution buffered at pH 4.8 by acetic acid/acetate to obtain a metal-to-ligand ratio that varied from 0:1 to 4:1. Different concentrations were screened, *i.e.*, $C_{\text{DOTA}} = 1 \times 10^{-4}$ M and $C_{\text{DOTA}} = 1 \times 10^{-3}$ M (final concentration). The UV–vis spectra were recorded, and the stoichiometry of the complex was determined by plotting the absorbance at the characteristic wavelength as a function of the metal-to-ligand ratios ($n(\text{Pb}^{2+})/n(\text{DOTA})$).

The same experiments were also conducted by collecting the ¹H NMR spectra of solutions buffered at pH 4.5 by acetic acid/acetate at different lead-to-DOTA molar ratios ($C_{\text{DOTA}} = 1 \times 10^{-3}$ M). Spectra were acquired using the same protocol described above.

Data Analysis. The equilibrium constants were obtained by refining the potentiometric and UV–vis spectrophotometric data using the PITMAP software.²⁷ All data refer to the equilibria $p\text{M}^{m+} + q\text{H}^+ + r\text{L}^{l-} \rightleftharpoons \text{M}_p\text{H}_q\text{L}_r^{pm+q-rl}$, where M is the metal ion and L the nonprotonated ligand. The constants for ligand deprotonation and Pb²⁺ hydrolysis were taken from the literature.^{20,28}

Pb²⁺-DOTA Dissociation Kinetics. The dissociation kinetics of the Pb²⁺-DOTA complex was studied under pseudo-first-order conditions in an aqueous solution at room temperature without control of the ionic strength by adding concentrated aqueous solutions of HCl (0.01, 0.1, and 1 M) to aqueous solutions of the preformed complex. The concentration of the Pb²⁺-DOTA complex after dilution was 1×10^{-4} M.

The dissociation reaction was followed by the intensity of the absorption band of the complexes decreasing over time. The spectroscopic data were processed to obtain a $\ln A_t$ versus t and fitted using the equation $\ln A_t = \ln A_0 - {}^d k_{\text{obs}} \cdot t$ where A_t and A_0 are the absorbances at time t and at the beginning of the reaction, respectively, and ${}^d k_{\text{obs}}$ is the observed dissociation rate constant. Each measurement was repeated at least in triplicate.

CONCLUSION

Herein, we have reported a thermodynamic reinvestigation of the Pb²⁺-DOTA complexes through a combination of pH potentiometry, UV–vis spectroscopy, and NMR spectroscopy, demonstrating that the speciation obtained in the previous studies was uncorrected and that the corresponding formation constants were underestimated. The involvement of 2:1 metal-to-ligand species considered in the case of Chaves *et al.*, and the complicated ternary system employed for the measurements by Pippin *et al.* (who studied solutions containing Pb²⁺, DOTA, and EDTA), might at least in part explain the observed differences. This work provides revised and accurate thermodynamic data for the very relevant Pb²⁺-DOTA complexes, which should be used as a new stability benchmark during the development of lead chelators.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c00387>.

Example fitting of potentiometric titrations, UV–vis spectra at equilibrium conditions, UV–vis and NMR spectra at constant pH and various metal-to-ligand ratios, UV–vis spectra showing the complexation and decomplexation kinetics between Pb^{2+} and DOTA, decomplexation fittings for the determination of $^d k_{\text{obs}}$, mono- and bidimensional NMR spectra of Pb^{2+} -DOTA solutions at equilibrium conditions at various temperatures, example of fitting of NMR titrations, half times, and $^d k_{\text{obs}}$ values (PDF)

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Notes

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