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Kinetics of Ga(NOTA) Formation from Weak Ga-Citrate Complexes

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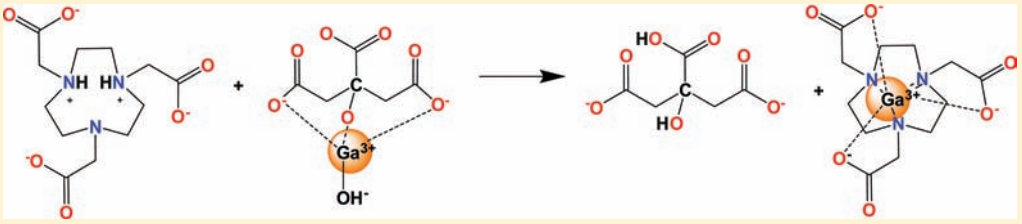
Supporting Information

ABSTRACT:



Gallium complexes are gaining increasing importance in biomedical imaging thanks to the practical advantages of the ^{68}Ga isotope in Positron Emission Tomography (PET) applications. ^{68}Ga has a short half-time ($t_{1/2}$ = 68 min); thus the ^{68}Ga complexes have to be prepared in a limited time frame. The acceleration of the formation reaction of gallium complexes with macrocyclic ligands for application in PET imaging represents a significant coordination chemistry challenge. Here we report a detailed kinetic study of the formation reaction of the highly stable Ga(NOTA) from the weak citrate complex (H_3NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid). The transmetalation has been studied using ^{71}Ga NMR over a large pH range (pH = 2.01−6.00). The formation of Ga(NOTA) is a two-step process. First, a monoprotonated intermediate containing coordinated citrate, GaHNOTA(citrate)*, forms in a rapid equilibrium step. The rate-determining step of the reaction is the deprotonation and slow rearrangement of the intermediate accompanied by the citrate release. The observed reaction rate shows an unusual pH dependency with a minimum at pH 5.17. In contrast to the typical formation reactions of poly(amino carboxylate) complexes, the Ga(NOTA) formation from the weak citrate complex becomes considerably faster with increasing proton concentration below pH 5.17. We explain this unexpected tendency by the role of protons in the decomposition of the GaHNOTA(citrate)* intermediate which proceeds via the protonation of the coordinated citrate ion and its subsequent decoordination to yield the final product Ga(NOTA). The stability constant of this intermediate, log $K_{GaHNOTA(citrate)*}$ = 15.6, is remarkably high compared to the corresponding values reported for the formation of macrocyclic lanthanide(III)-poly(amino carboxylates). These kinetic data do not only give mechanistic insight into the formation reaction of Ga(NOTA), but might also contribute to establish optimal experimental conditions for the rapid preparation of Ga(NOTA)-based radiopharmaceuticals for PET applications.

INTRODUCTION

Three radioisotopes of gallium (^{66}Ga, ^{67}Ga, ^{68}Ga) have attractive nuclear properties for medical use.[1] ^{67}Ga has been applied as gallium citrate for over 30 years as an alternative to $^{99\,m}$Tc for Single Photon Emission Computed Tomography (SPECT) imaging, though lately it has been proved that the citrate does not prevent the transchelation of the Ga^{3+} ion to biomolecules such as transferrin.[2] The two other isotopes are positron emitters and can be used in Positron Emission Tomography (PET) imaging for different diagnostic purposes considering their very different half-times (68 min for ^{68}Ga and 9.49 h for ^{66}Ga), decay and the energy of the particles emitted.[3] In contrast to ^{66}Ga and ^{67}Ga which are cyclotron products, ^{68}Ga is available from a commercial ^{68}Ge/^{68}Ga generator, which largely reduces the production costs and makes this PET isotope easily accessible.[4]

The Ga^{3+} ion has specific characteristics which render its aqueous coordination chemistry difficult. Because of its strong tendency for hydrolysis, in neutral aqueous media gallium(III) can exist only as insoluble hydroxides or in a strongly chelated form. The precipitation of $Ga(OH)_3$ starts already under acidic conditions, from pH ∼3.3,[5] though at nanomolar concentration, typically used in radiopharmaceutical applications, no precipitation occurs.[2] A safe biomedical use of Ga^{3+} requires chelating agents which prevent interaction of the metal with hydroxide but also with endogenous ligands which can transport the isotope to the area of biological interest. The ligands have to form gallium complexes of high stability and kinetic inertness to avoid any ligand exchange or transmetalation in the biological medium. In the context of biomedical imaging, macrocyclic poly(amino carboxylates) have been widely used for metal complexation, including $^{68}Ga^{3+}$ for PET applications.[6] These ligands are known to form thermodynamically highly stable complexes with various metal ions, like Ga^{3+} and lanthanides, which also show good kinetic inertness. Derivatives of H_4DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and H_3NOTA

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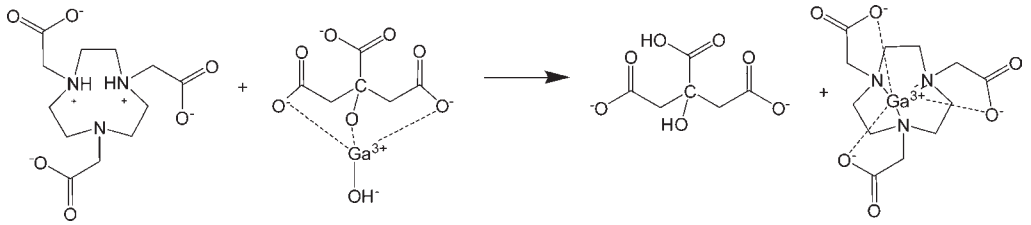
Scheme 1. Formation Kinetics of Ga(NOTA) Studied via Monitoring the Transfer of Ga^{3+} from the Citrate- to the NOTA-Complex. [a]



[a] The protonation states indicated apply to pH 5.0.

(1,4,7-triazacyclononane-1,4,7-triacetic acid), grafted to molecules of biological interest have been commonly applied as vectors of ^{68}Ga with promising results.[3] For instance, ^{68}Ga-NOTA-RGD was produced rapidly with a high yield and showed favorable properties like high stability, affinity and specificity for angiogenesis PET imaging.[7] The nine-member macrocyclic NOTA is known to form a Ga^{3+} complex of particularly high stability (log $K_{Ga(NOTA)}$ = 31.0).[6] This is related to the smaller size of its macrocyclic cavity as compared to the twelve-member DOTA, which is not well adapted to the small Ga^{3+} ion (63 pm).[8]

Given the relatively short half-life of ^{68}Ga, fast formation kinetics is required for the preparation of the complexes. In the literature, various conditions including the use of different buffers, pHs, and temperatures have been reported for radiolabeling of DOTA- or NOTA-derivatives.[9] The formation reactions between these macrocyclic chelates and various metals, such as lanthanide ions but also Ga^{3+}, are typically slow. The acceleration of the complexation between the radiometal and the chelating agents would be highly desirable since the optimization of the labeling conditions could provide a considerable gain in the time window available for imaging. Generally speaking, kinetic data on Ga^{3+} complexes are rather scarce, mainly because of the difficulties associated with the strong hydrolysis of Ga^{3+}. Indeed, to study the complexation reactions of Ga^{3+} at pH > 3, the metal must be prechelated in the form of a weak complex. While dissociation kinetic data have been reported on Ga^{3+} chelates, including Ga(NOTA),[10,11] to the best of our knowledge, no detailed formation kinetic study has been published on Ga^{3+} macrocyclic complexes. The objective of the present work was to investigate the kinetics of Ga(NOTA) formation via ligand exchange from the weak gallium citrate complex over a large pH range (Scheme 1). Citrate has been chosen as a precomplexing agent since it is probably the most often applied buffer in the radiolabeling procedures with $^{68}Ga^{3+}$. Its role, however, is not limited to buffer the system, but it also behaves as a weak chelator to avoid hydrolysis and formation of insoluble $Ga(OH)_3$ in the preparation of radiopharmaceuticals. On the other hand, the choice of NOTA is explained by the utmost importance of high stability of its Ga^{3+} complex in biomedical applications. In addition, this ligand allows monitoring the formation reaction by ^{71}Ga NMR, which is only applicable for highly symmetrical Ga^{3+} species such as Ga(NOTA). For comparison, the formation kinetics of Ga(NOTA) from the weak acetate complex has been also assessed.

RESULTS AND DISCUSSION

The formation kinetics of Ga(NOTA) was studied by following the transchelation of gallium citrate or gallium acetate complexes with NOTA at different pHs and 25 °C. ^{71}Ga NMR was used to monitor the time course of the ligand exchange. The high quadrupole moment of the ^{71}Ga nucleus results in a significant line-broadening, which limits the observation of the ^{71}Ga NMR signal to few, very symmetric species, such as $[Ga(H_2O)_6]^{3+}$, $[Ga(OH)_4]^-$, or Ga(NOTA); thus, no ^{71}Ga NMR signal can be detected for the citrate or acetate complexes.[12] We monitored the increase of the Ga(NOTA) signal in comparison to the signal of $[Ga(OH)_4]^-$ which is used as reference (Figure 1).

The presence of acetate and citrate prevents the formation of insoluble Ga^{3+} hydroxides. The absence of the hydroxide precipitate allows studying the kinetics of the metal transfer from the citrate or acetate to the NOTA complex over a large pH range, between pH 2 and 6. The excess of the exchanging NOTA ligand ensures pseudo-first order conditions; thus, the rate of the Ga(NOTA) complex formation may be described by eq 1:

$$\frac{d[GaNOTA]}{dt} = k_{obs}[Ga]_0 \quad (1)$$

where $[Ga]_0$ is the total concentration of free Ga^{3+} and k_{obs} is the pseudo-first order rate constant. The formation reaction was investigated at different pH values and with varying concentrations of NOTA for three different citrate concentrations. For all systems and at all pHs, k_{obs} was found to increase with the NOTA concentration, showing saturation curves (Figure 2 and in Supporting Information, Figure S7).

Under the experimental conditions and at pH between 4.5 and 6.3, in the Ga^{3+}-citrate system the major species is $[Ga(citrate)OH]^-$ (log β_{MLOH} = 7.1), while $[Ga(citrate)_2]^{3-}$ (log β_{ML2} = 15.0) appears only at a citrate excess.[5] In these experiments, in addition to the citrate, acetate is used as a buffer in much higher concentration (0.1 M) than citrate. Acetate ions can also form weak complexes with Ga^{3+}. On the basis of literature data,[13] we find that two species are predominant in the Ga^{3+}-acetate system in the pH range of our study: $[Ga_2(OH)_2Ac]^{2+}$ is the major species (log $\beta_{M2(OH)2\ L}$ = −1.16), while $[GaAc]^{2+}$ is present in lower concentration (log β_{ML}= 2.41). However, citrate complexes are considerably more stable and in the presence of citrate, no acetate complexes form (see species distribution curves in Supporting Information).

Although the main purpose of this work was to assess the formation kinetics of Ga(NOTA) in the presence of citrate, for comparison, we have also performed a formation kinetic study in a more limited pH range in solutions containing only acetate. Like in the presence of citrate (Figure 2), saturation curves were obtained with increasing NOTA concentration in the samples with only acetate as well. Figure 3 compares the reaction rate

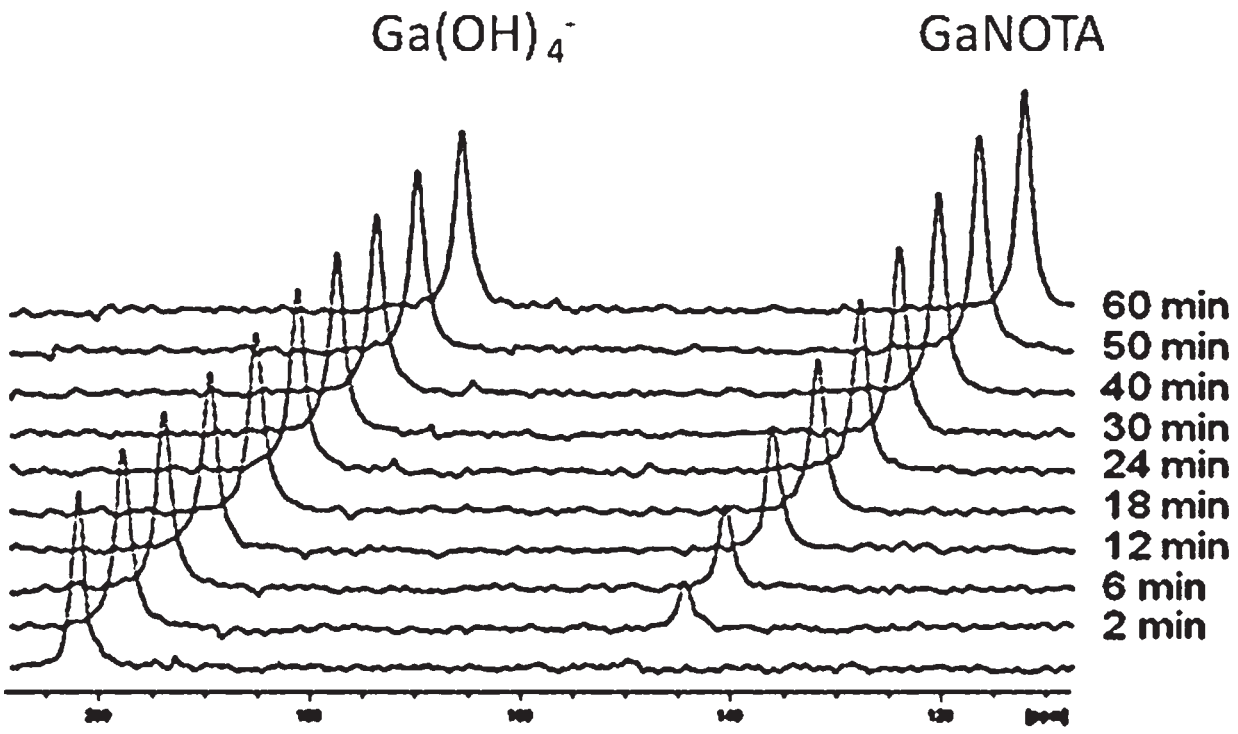


Figure 1. Representative ^{71}Ga NMR spectra in the formation reaction of Ga(NOTA) from Ga-citrate. c_{Ga} = 4 mM, c_{citr} = 4.7 mM, c_{NOTA} = 40 mM, pH = 4.51 (0.1 M acetate buffer), I = 1 M $NaNO_3$, 25 °C. The spectra were recorded in a 10 mm NMR tube which contains a 5 mm insert with a 0.1 M $Na[Ga(OH)_4]$ solution as reference.

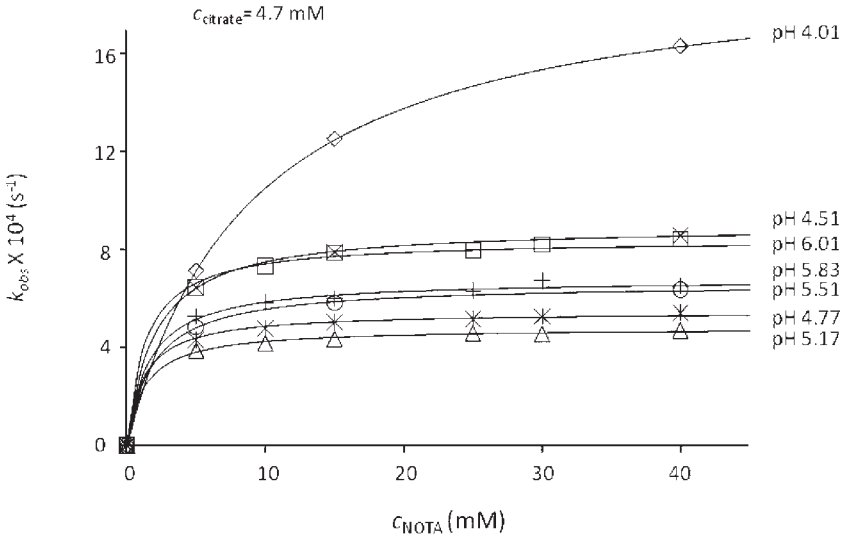


Figure 2. Representative series of pseudo-first-order rate constants, k_{obs}, as a function of NOTA concentration. c_{Ga} = 4 mM. The curves represent the fit to the data points as explained in the text.

constants in acetate only with those measured in solutions containing citrate and acetate. The absolute values of the observed reaction rate constants obtained in the presence of acetate only are considerably lower than those obtained in the presence of citrate, showing the very important role of citrate in accelerating the kinetics of Ga(NOTA) formation. The effect of citrate to accelerate the formation of Ga(NOTA) is strongly dependent on the pH, as clearly visible even in the relatively limited pH range available for comparison between the studies with or without citrate. While at pH 5.17 the k_{obs} values differ only by a factor of 2−2.5, at pH 4.5, the reaction rate constants are about 10 times higher in the presence of citrate than in its absence (Figure 3).

The comparison reveals another surprising phenomenon. Without citrate, the formation rate of Ga(NOTA) increases with increasing pH, as it is typically observed in the formation reactions of NOTA- or DOTA-complexes.[14] This behavior is related to the OH^--mediated deprotonation of the protonated intermediate (see below). In the presence of citrate, the rate

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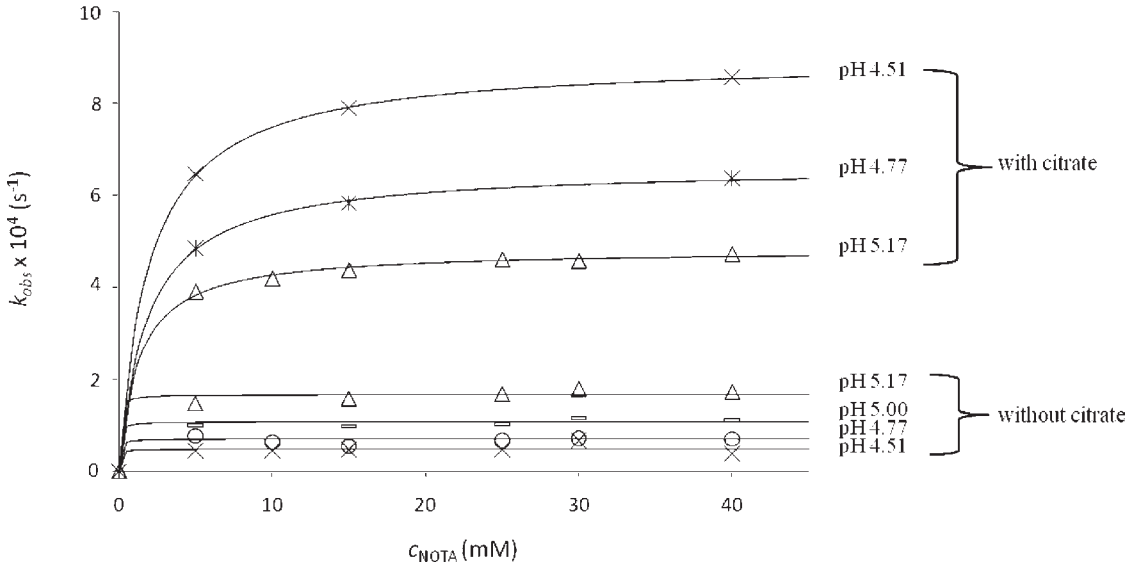

Figure 3. Comparison of the observed pseudo-first-order rate constants, k_{obs}, as a function of NOTA concentration, in the absence and in the presence of 4.7 mM citrate, at pH = 5.17 (△); 5.00 (−); 4.77 (○), 4.51 (×), c_{Ga} = 4 mM, $c_{acetate}$ = 0.1 M. The curves represent the fit to the data points as explained in the text.

Scheme 2

$$Ga^{3+} + H_iNOTA \underset{}{\overset{K^*}{\rightleftharpoons}} [GaHNOTA]^* \xrightarrow{k} GaNOTA + H^+$$

constants show this usual tendency only between pH 6.01 and pH 5.17 under our experimental conditions, while at pH below 5.17, the observed rate constants strongly increase with decreasing pH. This difference in the pH dependency of the reaction rates between the citrate-free and the citrate containing samples suggests a different mechanism of complex formation which will be assessed in the following sections.

Formation of Ga(NOTA) in the Absence of Citrate. The saturation curves observed for varying concentrations of NOTA are characteristic of a complex formation reaction proceeding in two steps: a rapid, equilibrium formation of an intermediate which is followed by the slow transformation of the intermediate to yield the final product in the rate-determining step (Scheme 2).[14]

In the literature, formation kinetic studies have been reported for LnNOTA complexes.[15] The intermediate has been identified as the monoprotonated LnHNOTA* complex which then deprotonates to yield the final LnNOTA chelate. The monoprotonated nature of the LnHNOTA* intermediate was evidenced by monitoring the pH variation occurring during the complex formation in a slightly buffered solution. Similarly to the pH dependency of the formation rates observed for Ga(NOTA) in the absence of citrate (Figure 3), the rates of formation of the LnNOTA complexes also increased with increasing pH.

In analogy to the LnNOTA complex formation, we can assume that, in the absence of citrate, the formation reaction of Ga(NOTA) proceeds via a two-step process. The intermediate is supposed to be the monoprotonated complex, GaHNOTA*, with a stability constant as defined in eq 2:

$$K_{GaHNOTA^*} = \frac{[GaHNOTA^*]}{[Ga][HNOTA]} \quad (2)$$

In the intermediate, the proton is supposed to be attached to a macrocycle nitrogen, analogously to LnHNOTA*, LnH_2DOTA^* or LnH_2TRITA^* intermediates (H_4TRITA = 1,4,7,11-tetraazacyclotridecane-1,4,7,11-tetraacetic acid).[15−17] Electrostatic repulsion between the metal ion and this proton could preclude the Ga^{3+} from entering the macrocyclic cavity of the ligand during the initial stages of complex formation.

The rate-determining step is the deprotonation and the rearrangement of the intermediate followed by the entrance of the metal ion into the macrocycle cage:

$$\frac{d[GaNOTA]}{dt} = k_{obs}[Ga]_0 = k[GaHNOTA^*] \quad (3)$$

The concentration of the noncomplexed ligand can be described by eq 4 using the protonation constants of NOTA, where the fully deprotonated form can be neglected in the pH range of the study.

$$[NOTA]_{free} = [HNOTA](1 + K_2[H^+] + K_2K_3[H^+]^2) = \alpha[HNOTA] \quad (4)$$

The total metal concentration can be expressed by

$$[Ga]_0 = [GaHNOTA^*] + [Ga]_{free} \quad (5)$$

Then k_{obs} can be deduced as in eq 6:

$$k_{obs} = \frac{k \times \left(\frac{K_{GaHNOTA^*}}{\alpha}\right)[NOTA]}{1 + \left(\frac{K_{GaHNOTA^*}}{\alpha}\right)[NOTA]} \quad (6)$$

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Table 1. Stability Constants, $K_{GaHNOTA*}$, of the Reaction Intermediates in the Formation of LnNOTA and Ga(NOTA) Complexes

M	log $K_{MHNOTA*}$
Ga^{3+}	4.2 ± 0.3
Ce^{3+}	3.2[a]
Gd^{3+}	3.6[a]
Er^{3+}	3.8[a]

[a] Ref 15.

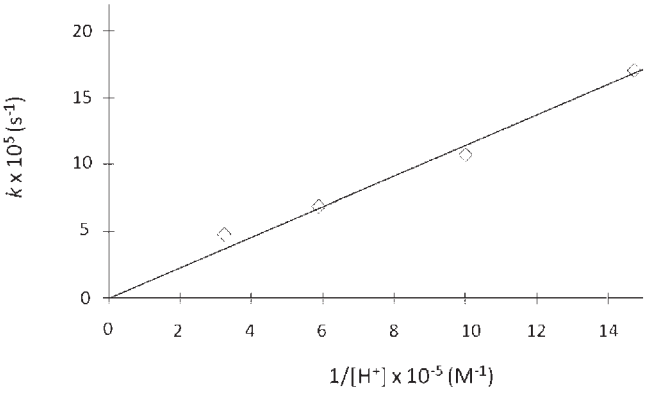

Figure 4. Formation rate constants for Ga(NOTA), without citrate, as a function of $1/[H^+]$, at 25 °C. The curve represents the fit of the data points to eq 7 as explained in the text.

The pseudo-first-order rate constants measured at various pHs were fitted to eq 6, and the stability constant of the intermediate, $K_{Ga(NOTA)H*}$, and the rate constants, k, were calculated. Table 1 shows the stability constant of the monoprotonated intermediate in comparison to those calculated for the corresponding Ln^{3+} analogues. The stability constant obtained for the GaHNOTA* intermediate is higher than those calculated for the Ln^{3+} analogues. This difference reflects the higher stability constant of Ga(NOTA) with respect to those of LnNOTA complexes ($\log K_{Ga(NOTA)} = 31.0$ vs $\log K_{GdNOTA} = 13.7$).[18] On the other hand, we have to note that the stability constant calculated for the GaHNOTA* intermediate is still a conditional constant since the [Ga] concentration in eq 2 includes all Ga^{3+} species not complexed to NOTA (in the form of acetate or eventually hydroxo complexes).

The rate constants, k, are inversely proportional to the $[H^+]$ concentration (Figure 4). This implies an OH^- catalyzed deprotonation process of the intermediate, as described by eq 7.

$$k = k_0 + k' \times 1/[H^+] = k_0 + k_{OH}[OH^-] = k_0 + k'/K_w[OH^-] \quad (7)$$

The k values versus the $[OH^-]$ concentration have been fitted to eq 7. When fitting k_0, we obtained a very small value with a large error; therefore k_0 was fixed to 0 and the k_{OH} value obtained is given in Table 2. The zero intercept of the straight line in Figure 4 shows that the spontaneous transformation of the monoprotonated intermediate is very slow and negligible under our experimental conditions. The dominant pathway over this pH range is the OH-catalyzed deprotonation of [GaHNOTA]*. The formation of Ga(NOTA) via this pathway, however, is considerably slower than the formation of the Ln^{3+} analogues, as shown by the 2 orders of magnitude difference in the k_{OH} values between Ga(NOTA) and GdNOTA (Table 2).

Table 2. Formation Rate Constants of the Complexes

	Ga(NOTA) in the presence of citrate[a]	Ga(NOTA) in the absence of citrate[a]	GdNOTA[b]
k_{OH} (M^{-1} s^{-1})	(4.2 ± 0.9) × 10^4	(1.14 ± 0.04) × 10^5	7.1 × 10^7
k_0 (s^{-1})	(4.3 ± 0.5) × 10^{-4}	0	
k_1 (M^{-1} s^{-1})	9 ± 2		
k_2 (M^{-2} s^{-1})	(9.6 ± 0.6) × 10^4		

[a] This work. [b] Ref 15.

Formation of Ga(NOTA) in the Presence of Citrate. In the presence of citrate, the pH dependency of the observed pseudo-first-order rate constants is different from that observed in the absence of citrate and follows an unexpected trend. At any of the three different citrate concentrations, the plateau of the saturation curves shows a minimum at pH 5.17, and then increases for higher and also for lower pH values. We should note that there is no significant difference between the three citrate concentrations, thus the citrate has no effect on the kinetics in the concentration range studied. As in the absence of citrate, the saturation curves in Figure 2 imply a fast equilibrium formation of an intermediate, followed by its transformation in a slower, rate-determining step to yield the final product. To assess the nature of the intermediate, we have monitored the pH variation in the time course of the reaction in slightly buffered samples (0.01 M acetate), upon mixing a solution containing Ga^{3+} and citrate (5×10^{-4} M, each, 5 mL) with a solution of $H_{2.04}$NOTA (5 mL, 5×10^{-4} M) at a starting pH 4.27 (at this pH and concentration, the NOTA ligand has an average protonation of 2.04). Immediately after mixing the two solutions, the pH shows a slight increase to pH 4.33, which is followed by a successive, slow increase to pH 4.39 (within 1 day). The pH variation along the formation reaction has been found reproducible in several experiments. Because of the protonation equilibria involving also the citrate ion, the situation is more complicated than it was for the analogous experiment with LnNOTA,[15] LnDOTA,[16] or LnTRITA[17] complexes. Nevertheless, in the second step, the pH would be clearly expected to decrease if this second step indeed corresponds to the deprotonation of the monoprotonated intermediate. The observation of an opposite pH change is not compatible with the classical complex formation mechanism involving a monoprotonated intermediate. On the basis of this result, we hypothesize that the intermediate also contains a citrate ligand that remains coordinated to the Ga^{3+} ion (GaHNOTA(citrate)*) and the rate determining step is the deprotonation of this intermediate accompanied by the release of the citrate. The citrate released in this second step will become partially protonated ($\log K_{H1} = 5.70$, $\log K_{H2} = 4.35$, $\log K_{H3} = 2.91$) which explains the pH increase observed (Scheme 3). Although we do not have any direct evidence of the nature of the intermediate, the suggested composition is the most plausible explanation to account for the pH variation observed.

We should note that the assumption of an intermediate that contains coordinated hydroxide might also lead to the observed pH increase in the second step. However, we refuted this hypothesis since, if it was the case, a similar intermediate with similar behavior should be observed also in the absence of citrate.

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Scheme 3

$$Ga(citrate) + HNOTA \overset{K^*}{\rightleftharpoons} [GaHNOTA(citrate)]^* \xrightarrow{k\ /\ +H^+} GaNOTA + H_i(citrate) \quad i>1$$

The stability constant of GaHNOTA(citrate)* is defined as

$$K_{GaHNOTA(citrate)^*} = \frac{[GaHNOTA(citrate)^*]}{[Ga][HNOTA][citrate]} \quad (8)$$

Considering that the transformation of the intermediate is the rate-determining step of the complex formation, the rate of the pseudo-first-order reaction can be described as

$$\frac{d[GaNOTA]}{dt} = k_{obs}[Ga]_0 = k[GaHNOTA(citrate)^*] \quad (9)$$

The total metal concentration can be expressed by

$$[Ga]_0 = [GaHNOTA(citrate)^*] + [Ga]_{free} \quad (10)$$

where $[Ga]_{free}$ is the total metal concentration not complexed to NOTA. Under our conditions, the concentration of gallium hydroxo complexes is negligible.

$$[Ga]_{free} = Ga\left(1 + \beta_{Ga(citrate)}[citrate]_{free} + \beta_{Ga(citrate)_2^{3-}}[citrate]_{free} + \frac{\beta_{Ga(citrate(OH)^-}[citrate]_{free}}{[H^+]}\right) = \beta[Ga] \quad (11)$$

As above, the concentration of the noncomplexed ligand can be described by eq 4, and the concentration of the noncomplexed citrate can be expressed by eq 12, using the corresponding protonation constants.

$$[citrate]_{free} = [citrate](1 + K_{Hcitrate}[H^+] + K_{Hcitrate}K_{H_2citrate}[H^+]^2 + K_{Hcitrate}K_{H_2citrate}K_{H_3citrate}[H^+]^3) = \delta[citrate] \quad (12)$$

Then, k_{obs} can be deduced as in eq 13

$$k_{obs} = \frac{kK_{GAHNOTA(citrate)^*}[citrate][NOTA]/\alpha\beta\delta}{1 + K_{GaHNOTA(citrate)^*}[citrate][NOTA]/\alpha\beta\delta} \quad (13)$$

The pseudo-first-order rate constants were fitted to eq 13 at each pH and at the three different citrate concentrations (4.7 mM, 9.8 mM, and 19 mM). The rate constants, k, and the stability constant of the intermediate, $K_{GaHNOTA(citrate)}{}^*$, were calculated (Table 3).

At the various pHs and citrate concentrations, very similar values have been obtained for the stability constant of the GaHNOTA(citrate)* intermediate. The stability constant is remarkably high, however, since the stoichiometry of the intermediate is supposed to be different from that in the absence of citrate or that detected in LnNOTA formation, we cannot directly compare this value to the stability constants of those intermediates. On the other hand, high stability is expected for GaHNOTA(citrate)* since the Ga^{3+} ion has a complete coordination sphere with an overall coordination number of CN = 6, involving the three carboxylates of the NOTA, as well as two carboxylates and one hydroxyl from the citrate. Since the typical coordination number is 6 in Ga^{3+} complexes, the

Table 3. Stability Constants of the Reaction Intermediate, $K_{GaHNOTA(citrate)}{}^*$, and the Rate Constants, k, Calculated for the Different pH Values and Citrate Concentrations

citrate (mM)	pH	log $K_{GaHNOTA(citrate)^*}$	$k \times 10^4$ (s^{-1})
4.7	6.00	16.0	8.8 ± 0.3
	5.83	15.9	6.9 ± 0.3
	5.51	15.8	5.6 ± 0.3
	5.17	15.7	4.9 ± 0.3
	4.77	15.6	6.7 ± 0.4
	4.51	15.6	9.0 ± 0.4
	4.03	14.9	19.9 ± 0.8
9.8	6.00	16.7	8.0 ± 0.5
	5.17	15.5	5.5 ± 0.5
	4.77	15.7	6.3 ± 0.4
	4.02	14.9	19.5 ± 0.9
19.0	6.00	15.8	9.4 ± 0.6
	5.17	15.9	5.7 ± 0.6
	4.77	15.5	8.4 ± 0.5
	4.03	15.0	22.4 ± 0.9
		average: 15.6 ± 0.7	

GaHNOTA(citrate)* intermediate is expected to be highly stable in comparison to LnHNOTA* intermediates in which the coordination number of the Ln^{3+} ion is very low (CN = 4) as compared to the preferred CN of lanthanide ions (CN = 8 or 9). Differences in the steric strain can be also important for the differences in the stability. In the intermediate, the citrate is expected to coordinate to the Ga^{3+} as a tripodal ligand with two deprotonated carboxylate donors and the alcoholic oxygen, as it was proved by an X-ray crystallographic study for the Ga-(citrate)$_2$ complex.[19]

The variation of the plateau of the k_{obs} versus c_{NOTA} curves (Figure 2) as a function of pH has already shown the unusual pH dependency of the complex formation reaction, which is confirmed by the pH dependency of the k values (Table 3). When they are plotted against the inverse proton concentration, $1/[H^+]$, they show a curve with a minimum for all three citrate concentrations (Figure 5). This is in contrast with the results obtained in the absence of citrate (Figure 4) and with all previous observations of complex formation reactions of LnDOTA or LnNOTA analogues where a linear dependency of k on $1/[H^+]$ was reported.

To explain this unusual pH dependency of the k values, we have to consider the transformation of the intermediate which was assumed to be the ternary complex involving the coordination of the Ga^{3+} ion to a citrate ion and to the NOTA protonated on the macrocyclic nitrogen. The formation of the final Ga-(NOTA) complex implies the protonation of the citrate ion followed by its immediate dissociation and the deprotonation of the macrocycle nitrogen. These two processes might occur simultaneously or subsequently. The protonation of the coordinated citrate is expected to be catalyzed by protons, while the

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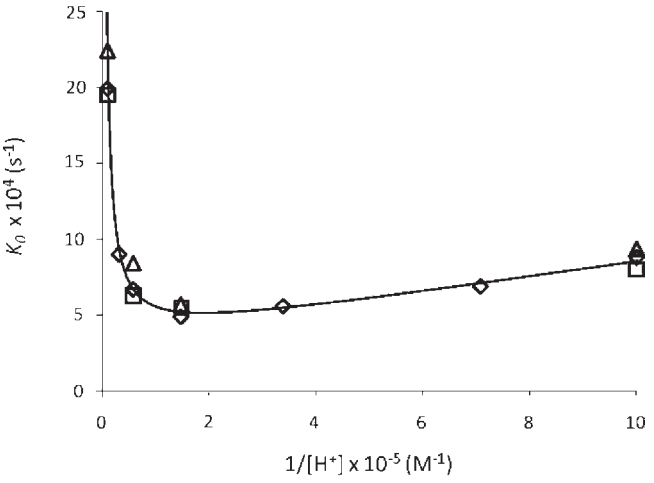
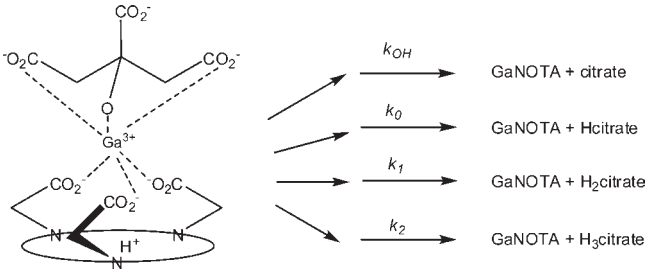


Figure 5. Formation rate constants for Ga(NOTA), as a function of $1/[H^+]$, 25 °C, measured at different citrate concentrations, $c_{citrate}$ = 4.7 mM (◇), 9.8 mM (□), and 19.0 mM (△). The curve represents the fit to all data points as explained in the text.

Scheme 4. Intermediate, Assumed to Be a Ternary Complex Involving Citrate Coordination, May Be Transformed via Spontaneous and OH^-- or H^+-Catalyzed Pathways



deprotonation of the macrocycle nitrogen is a hydroxide-catalyzed process. In addition to the H^+ or OH^- catalyzed pathways, a spontaneous transformation of the intermediate might also occur. On the basis of this hypothesis, the rate determining step can involve the following pathways depending on the pH, as shown in Scheme 4.

According to the observed pH dependency of the k values (Figure 5), the first pathway, catalyzed by OH^- ions, is predominant for pH values above 5.17 where the formation rate of the product, k, is inversely proportional to the proton concentration. The last two pathways, involving the k_1 and k_2 rate constants, imply a H^+ catalyzed dissociation of the intermediate. At increasing proton concentration, protonation of the citrate anion occurs which will destabilize the intermediate, and lead to the dissociation of the citrate, followed by the deprotonation of the macrocyclic nitrogen and the subsequent entering of the metal into the macrocyclic cavity.

The rate of formation of Ga(NOTA) from the intermediate can be then described by eq 14. The rate constants k versus $1/[H^+]$ as shown in Figure 5 have been fitted to eq 14, simultaneously for all three citrate concentration. The values of k_{OH}, k_0, k_1, and k_2 obtained in the fit are summarized in Table 2.

$$k = k_0 + k_{OH}[OH^-] + k_1[H^+] + k_2[H^+]^2 \qquad (14)$$

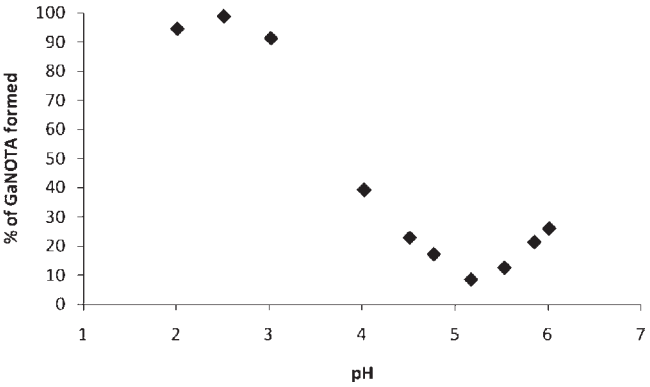


Figure 6. Percentage of the Ga(NOTA) complex formed after 2 min of reaction time as a function of pH. c_{Ga} = 4 mM, $c_{citrate}$ = 4.7 mM and c_{NOTA} = 40 mM, in 0.1 M acetate buffer, 25 °C.

These kinetic data clearly confirm two predominant processes depending on the pH: a base catalysis at above pH ∼5, described by k_{OH}, and a strong acid catalysis at lower pH, described by k_1 and k_2. The fit also resulted in a nonzero value for the k_0, which points to a relatively important contribution of the spontaneous transformation of the intermediate (Table 2).

At pHs below 3, the complex formation is too fast; thus, the reaction could not be followed, and the formation rates could not be calculated from the ^{71}Ga NMR measurements. Indeed, this NMR method is limited to relatively slow reactions since at least 1 min is needed to record the first ^{71}Ga NMR spectra after mixing the reactants. At low pH (<3), the reaction was almost complete in a few minutes; nevertheless, these data confirm that the formation becomes considerably faster with decreasing pH. To give a qualitative picture of the acceleration of the formation reaction of Ga(NOTA) with increasing proton concentration, Figure 6 shows the percentage of the Ga(NOTA) complex formed 2 min after mixing the Ga^{3+}-citrate and NOTA solutions. This empirical curve clearly shows that the formation rate of Ga(NOTA) is remarkably higher at pH 2−3 as compared to pH 5. Below pH 2.5, the formation rate seems to decrease with increasing acidity.

CONCLUSION

We have studied the kinetics of Ga(NOTA) formation from Ga^{3+}-citrate and Ga^{3+}-acetate complexes in a large pH-range. ^{71}Ga NMR was used to monitor the increase of the Ga(NOTA) concentration. We have evidenced that the use of citrate for precomplexation of the Ga^{3+} not only avoids hydroxide precipitation but also results in much higher formation rates than those observed in an acetate buffer without citrate. In the presence of citrate, the formation kinetics has an unexpected pH dependency. The reaction rates show a minimum at pH ∼5, then become faster with increasing or decreasing proton concentration. The first step of the reaction is the equilibrium formation of an intermediate which is monoprotonated on the macrocyclic nitrogen. In the presence of citrate, the intermediate is assumed to be a highly stable ternary complex involving also the coordination of a citrate ion GaHNOTA(citrate)*. In the second step, this intermediate can be transformed to yield Ga(NOTA) via both OH^-- or H^+-catalyzed pathways which accounts for the unusual pH-dependency of the reaction rates. We hypothesize that the H^+ catalysis is related to the protonation

of the coordinated citrate which leads to its decoordination and to the subsequent formation of the final complex. The effect of OH^- ions, classically observed in analogous metal complex formation reactions, can be explained by the OH- catalysis of the deprotonation of the macrocycle nitrogen. Even if not all mechanistic aspects can be directly proved, our experimental data clearly indicate that the Ga(NOTA) formation in the presence of citrate is faster at pH 2−3 than at higher pHs. These unexpected results, which have to be validated in radiochemical concentrations, might contribute to the development of more efficient radiolabeling strategies for ^{68}Ga or ^{67}Ga complexes used as radiopharmaceuticals in diagnostic applications.

EXPERIMENTAL SECTION

Sample Preparation. H_3NOTA and sodium citrate were purchased from Chematech and Aldrich, respectively. The $Ga(NO_3)_3$ solution was prepared by dissolving 99.99% Ga metal in HNO_3 (the final pH was 1.3). The Ga^{3+} concentration was determined by adding excess of Na_2H_2EDTA solution to the $Ga(NO_3)_3$ solution, and titrating back the Na_2H_2EDTA with Zn^{2+} at pH 5.8 in the presence of xylenol orange indicator. Solutions of NOTA were prepared by dissolving H_3NOTA in 1 M $NaNO_3$ and 0.2 M acetate buffer. Ga-citrate solutions were made by mixing a $Ga(NO_3)_3$ solution with solutions of sodium citrate (10 mM, 20 mM, 40 mM) to give a finale Ga/citrate ratio of 1.18:1, 2.45:1, and 4.75:1. The pH of all solutions was adjusted by adding 0.1 or 1 M HCl and NaOH. The final acetate concentration in the kinetic measurements was 0.1 M.

Kinetic Studies. The rates of Ga(NOTA) formation from Ga-citrate species were measured at 25 °C and at an ionic strength of 1 M $NaNO_3$. ^{71}Ga NMR spectra were recorded on a Bruker Avance 500 spectrometer (^{71}Ga, 152.5 MHz, 11.75 T). Chemical shifts were externally referenced to a 0.01 M $Ga(NO_3)_3$ solution in 0.1 M HNO_3; 0.0 ppm. Identical volumes of NOTA and Ga-citrate solutions were mixed in a 10 mm NMR tube wherein there was a reference (5 mm NMR tube) with a $Ga(NO_3)_3$ solution in 0.1 M NaOH. The kinetic experiments were performed at a constant temperature of 25.0 °C maintained by the NMR spectrometer and/or by a thermostatted bath. The pH was checked in each sample after the kinetic experiment, and even at the lowest pHs, where the acetate has no more buffer effect, its maximum change did not exceed 0.05.

The concentration of Ga^{3+} was 4 mM in all experiments; the concentration of citrate was 4.76, 9.8, or 19.0 mM, and the concentration of NOTA varied between 5 and 40 mM. To monitor the formation of the Ga(NOTA) complex, the intensity of the ^{71}Ga NMR signal was measured in 2 min intervals with respect to the signal of the reference. All fits of the kinetic data were performed with the program Micromath Scientist.[20]

ASSOCIATED CONTENT

Supporting Information. Table of literature data for protonation and stability constants of species involving Ga^{3+}, acetate, citrate, and NOTA. Species distribution diagrams. This material is available free of charge via the Internet at http://pubs.acs.org.

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