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Equilibrium and dissociation kinetics of the [Al(NOTA)] complex (NOTA = 1,4,7-triazacyclononane-1,4,7-triacetate)

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Abstract A detailed investigation of the equilibria and dissociation kinetics for the [Al(NOTA)] complex has been carried out. This complex and its derivatives are known as very good carriers for ^{18}F-isotope in positron emission tomography. The thermodynamic stability of [Al(NOTA)] has been studied by "out of cell" pH-potentiometric technique since the formation rate of the complex is very low in acidic medium. ^{1}H- and ^{27}Al-NMR spectra have been recorded to check the time course of equilibration and to validate the equilibrium model consisting of [Al(NOTA)] with $\lg K = 17.9(1)$ and [Al(HNOTA)]$^{+}$ with $\lg K^{H} = 1.9(3)$. A metastable mixed hydroxido complex [Al(NOTA)(OH)]$^{-}$ with $\lg K^{OH}_{Al(NOTA)} = -12.2(1)$ was detected in alkaline solution by direct pH-potentiometry, which transforms slowly to [Al(OH)$_4$]$^{-}$. The decomplexation reactions of [Al(NOTA)] have been investigated in both acidic and basic conditions. The rate of dissociation is extremely low in acidic medium, while in alkaline solution, it can be characterized by the rate law $k_{obs} = k_0 + k_1$ [OH^{-}], where $k_0 = (2.0 \pm 0.1) \times 10^{-6}$ s^{-1} and $k_1 = (6.8 \pm 0.5) \times 10^{-6}$ M^{-1}s^{-1}. The formation of the ternary [Al(NOTA)(F)]$^{-}$ complex via direct reaction of [Al(NOTA)] and F^{-} cannot be detected by either fluoride selective electrode or by ^{19}F-NMR spectroscopy. However, by applying solvent mixture (1:1 ethanol:water)

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and heating, the ternary [Al(NOTA)(F)]$^-$ complex was found to form quantitatively within 15 minutes.

Introduction

Macrocyclic amino-carboxylate ligands are widely used in clinical chemistry including medical diagnosis and therapeutic applications involving metal complexes. The ligands consisting of polyaza-macrocyclic rings and pendant arms show high selectivity if the size of the ring and the radius of the metal ion match perfectly. Moreover, the nature of the pendant arm attached to the N-atoms of the macrocycle can be used to tune the physico-chemical properties (stability and selectivity) of the metal complexes. In case of bifunctional ligands, there is also a conjugated biological vector (peptide, antibody etc.), i.e. a covalently bonded entity in the molecule, responsible for the selective targeting of the complexes in living organisms [1].

The most widely studied complexes are the MRI-contrast agents [1], where the macrocyclic ligands are 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA) and its derivatives, binding paramagnetic Gd(III) ions, while the 1,4,7-triazacyclononane-1,4,7-triacetate (NOTA) with a smaller cavity forms extremely stable complexes with smaller ions like Fe(III) or Ga(III) [2]. The interest in [Al(NOTA)] has recently renewed due to a new concept by D'Souza et al. [3–5], using [Al(NOTA)] as a ^{18}F-isotope carrier in positron emission tomography (PET). The preparation of [Al(NOTA)(^{18}F)]$^-$ is reported to be faster and simpler compared to the standard procedure for making ^{18}FDG (^{18}fluoro-deoxyglucose) used routinely in PET imaging. Targeted PET agents were developed recently using Al complexes formed with bifunctional NOTA-derivatives; very promising results (both in sensitivity and in resolution) have been reported in animals [3-5]. Radiochemists and medical researchers usually focus on radiochemical yields and in vivo stability, although one should know more about the chemical characteristics of the [Al(NOTA)] complex itself. In this paper, we deal with the physico-chemical characterization of [Al(NOTA)], by investigating and describing the complex stability constant and its dissociation in acidic and alkaline solutions using pH-potentiometry, ^{1}H- and ^{27}Al-NMR spectroscopy. Preliminary results on the identification of [Al(NOTA)(F)]$^-$ mixed complex by ^{19}F-NMR are also reported.

Experimental section

Materials and solution preparation

The stock solutions of 0.2 M AlCl$_3$, 0.1 M NaF and 0.03 M NOTA were prepared from solid AlCl$_3$·6H$_2$O, purchased from Reachim, NaF purchased from Sigma-Aldrich and solid NOTA, synthesized earlier in our group [6, 7].

Analysis

Concentrations of the $AlCl_3$ and NOTA solutions were determined by complex-ometric titration [8] and pH-potentiometry (see below), respectively.

Sample preparation

Owing to the slow formation of macrocyclic complexes, the stability constant of [Al(NOTA)] was determined by the out-of-cell method. 15 samples containing 3 mM NOTA and 3 mM $AlCl_3$ in 0.15 M NaCl were kept sealed at 37 °C in the pH-range 2–3, for 4 months. 3 separate samples of identical composition (at pH 2.0, 2.5 and 3.0) were followed by ^{1}H- and ^{27}Al-NMR to determine the time needed for complete equilibration at all pH values.

For investigating the kinetic properties of [Al(NOTA)] in both acidic and basic solutions, 1 mM [Al(NOTA)] in 1 M HCl and 0.05–1 M NaOH were used.

Samples used to determine the properties of [Al(NOTA)(OH)]$^-$ ternary complex using pH-potentiometric titrations contained 4 mM of [Al(NOTA)], 0.15 M of NaCl and 6.6 mM of HCl. Those used for investigating complex protonation contained 4 mM of [Al(NOTA)], 0.15 M of NaCl with starting pH set to 1.

The NMR samples were prepared by mixing Al(III), NOTA and F$^-$ in equimolar ratio (4 mM of $AlCl_3$, NaF and NOTA each, in acetate buffer at pH = 4) and heated at 100 °C for various time periods following the literature procedure of Dijkgraaf et al. [5]. Their ^{19}F-NMR spectra were recorded after an additional 24 h. The pH was adjusted by stepwise addition of 0.2 M NaOH and HCl solutions. An attempt to prepare [Al(NOTA)(F)]$^-$ was also undertaken by using solvent mixture (1:1 water:ethanol) as described by D'Souza et al. [3] for the preparation of single crystals using stable Na^{19}F ($AlCl_3$, NOTA and F$^-$ solutions were mixed in water and the appropriate amount of ethanol added lastly).

NMR experiments

400 MHz ^{1}H-, 376.5 MHz ^{19}F- and 104.3 MHz ^{27}Al-NMR spectra were collected using a Bruker DRX 400 (9.4 T) NMR spectrometer. Calibration was performed with the signal of TMS for ^{1}H-spectra (0 ppm), 10 mM slightly basic NaF for ^{19}F-spectra (0 ppm) and that of 10 mM $AlCl_3$ for ^{27}Al-spectra (0 ppm). The temperature was kept at 25 ± 0.1 °C with a Bruker VT-1000 thermo controller. As all samples were made in H_2O, closed capillaries of D_2O were inserted into each sample and used for deuterium lock. The spectra were analyzed with the Bruker WinNMR software package.

Potentiometry

Titrations were carried out with a *Metrohm 702SM Titrino* titration workstation with the use of a *Metrohm-6.0233.100* combined electrode. The titrated solutions (6.00 mL in total) were thermostated at 25 °C. The samples were stirred with a magnetic stirrer and N_2 gas was bubbled through the solutions before and during the procedure to avoid any

interaction with air. The titrations were performed in the pH-range of 2.2–12.05. The pH-electrode was calibrated with KH-phthalate (pH 4.005) and borax (pH 9.177) buffers. [H$^+$] concentrations were calculated from the measured pH values with the method proposed by Irving et al. [9]. Therefore, the equilibrium constants in this paper are stoichiometric constants. A 0.02 M HCl solution (in 0.15 M NaCl) was titrated with 0.2 M NaOH and the difference between the measured and calculated pH values were used to calculate the [H$^+$] from the pH values determined in the titration experiments. Equilibrium constants were calculated using the PSEQUAD program [10].

Results and discussion

Stability of [Al(NOTA)]

In order to characterize the Al(III)-NOTA system, first the protonation constants of NOTA^{3-} were measured using standard pH-potentiometric technique in 0.15 M NaCl (to mimic the conditions present in biological fluids). The ligand protonation constants could be defined as follows (Eq. 1):

$$L_{i-1} + H^+ \rightleftharpoons H_iL \tag{1}$$

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \, i = 1, 2, 3, 4 \tag{2}$$

The protonation constants of NOTA were calculated using the titration data and are shown in Table 1. These values are in a good agreement with the data obtained by Martell [2]. To the best of our knowledge, the stability constant of [Al(NOTA)] has not been determined yet. The formation equilibrium between NOTA^{3-} and Al(III)ion could not be studied by means of direct pH-potentiometry, because of the slow formation reaction of the complex in acidic medium. Hence, the so-called "out-of-cell" technique was used to determine the stability constant.

Using an estimated value for the stability constant of [Al(NOTA)] (lg$K \sim 17$), 15 separate samples were prepared within the pH-range of 2–3, changing the

Table 1 Protonation constants of the NOTA ligand and equilibrium constants of [Al(NOTA)] (25 °C, 0.15 M NaCl)

lgK_1^H	11.79(1)	11.96[c]
lgK_2^H	5.84(1)	5.65[c]
lgK_3^H	3.36(1)	3.17[c]
lgK_4^H	1.98(1)	–
lg$K_{Al(NOTA)}$	17.9(1)[a]	–
lg$K_{Al(NOTA)}^H$	1.9(3)[a]	–
lg$K_{Al(NOTA)}^{OH}$	−12.2(1)[b]	–

[a] Stoichiometric equilibrium constants by "out of cell" pH-potentiometry

[b] Calculated equilibrium constant of the metastable species, from "fast titration"

[c] 0.1 M KCl [2]

quantity of added strong acid. Having no information about the formation rate of [Al(NOTA)], three NMR samples (being in the pH range of 2–3) were also prepared to follow the process in time by using ^{1}H- and ^{27}Al-NMR techniques (Fig. 1). NMR spectra were recorded several times until no further changes could be observed. The time required for this was 4 months, after which the 15 "out of cell" samples were opened and their equilibrium pH values were measured. These data were used for equilibrium calculations with PSEQUAD.

Typical spectral changes can be seen in Fig. 1. The signal intensity of free Al(III) at 0 ppm ion decreased, while that of [Al(NOTA)] at 49 ppm increased with time. No further changes were observed after 4 weeks. Equilibration time was longer at lower pH-values. Therefore, the samples were aged for 4 months before opening and measuring their equilibrium pH-values. ^{1}H-NMR spectra showed mostly the free ligand signals after one week, which were replaced with those of the complex, with a more complicated pattern in the aged sample, in accordance with the conversion seen in the [27]Al spectra. When the equilibrium was reached, the pH-values for each separate sample were measured and their ^{1}H- and ^{27}Al-NMR spectra were also recorded (Fig. 2).

The signals in the ^{1}H-NMR spectra are quite difficult to assign, but the complex formation process is clearly shown in the range of 2.8–4.0 ppm with the increase of pH. This phenomenon can easily be followed with ^{27}Al-NMR: the signal intensity of the aqua complex $[Al(H_6O)]^{3+}$ at 0 ppm decreases, while that of [Al(NOTA)] at 49 ppm increases with pH. The ^{27}Al-NMR signal of [Al(NOTA)] is unexpectedly narrow, which can be explained by the compact and symmetric (octahedral) structure of the complex as evidenced by X-ray crystallography [11]. The stability constant of [Al(NOTA)] was
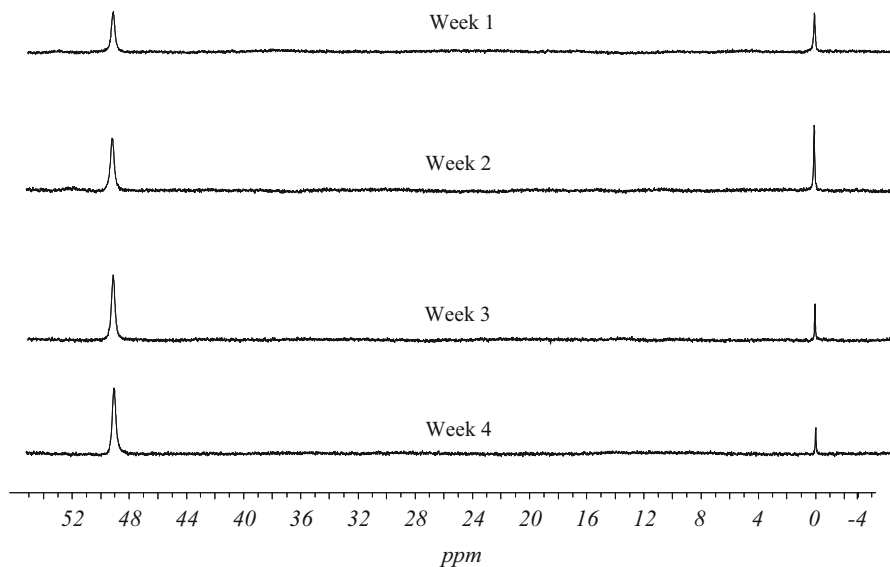

Fig. 1 104.3 MHz ^{27}Al-NMR spectra of the Al(III)-NOTA system ($c_{Al(III)}$ = 2.88 mM, c_{H3NOTA} = 2.99 mM, final pH = 2.79) as function of time

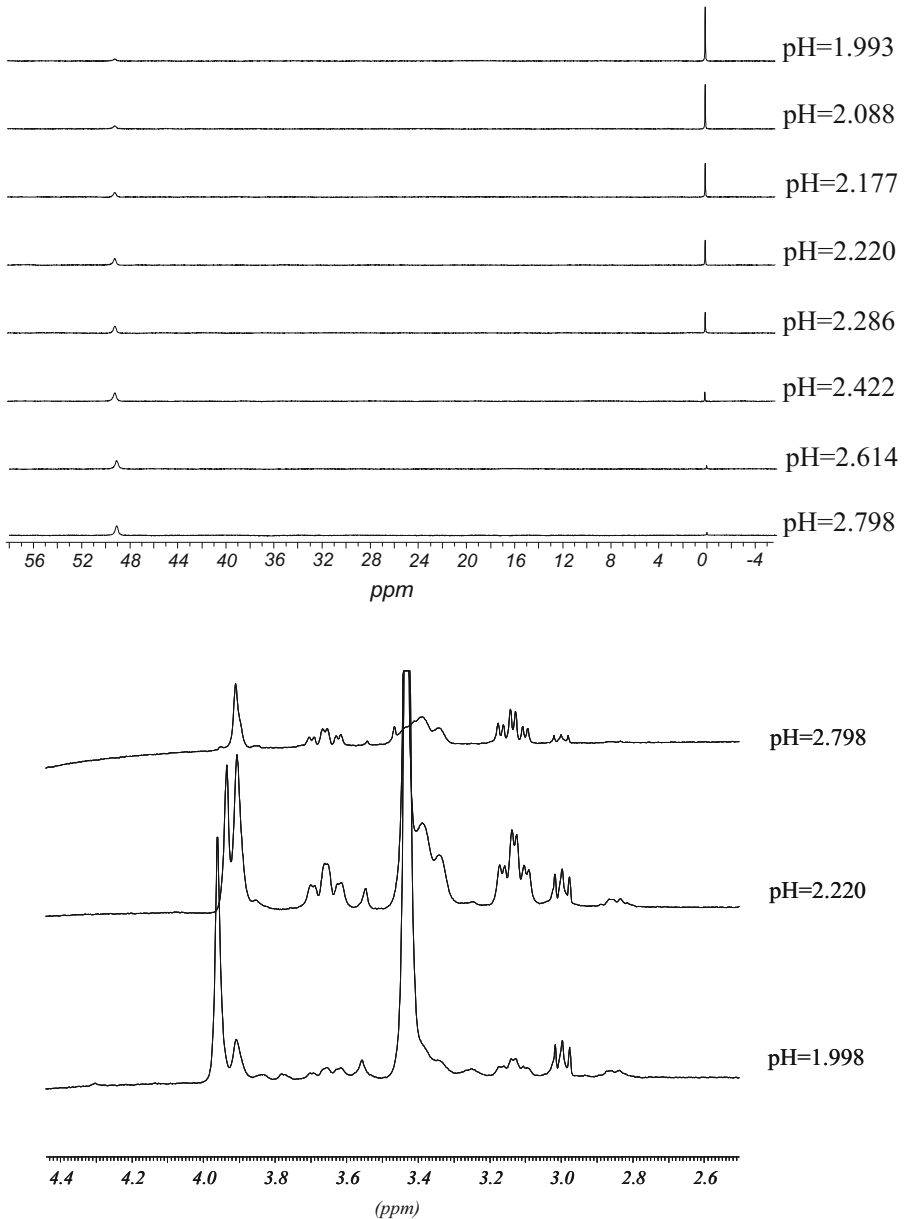**Fig. 2** 104.3 MHz ^{27}Al-(*top*) and 400 MHz ^{1}H-NMR (*bottom*) spectra of the Al(III)-NOTA system ($c_{Al\,(III)}$ = 2.88 mM, c_{H3NOTA} = 2.99 mM) at different pH values (aging time: 4 months)

calculated from the pH-metric data using PSEQUAD. The standard deviation of the obtained value as well as the fitting parameter decreases significantly when the formation of a monoprotonated species ($[Al(HNOTA)]^+$) was also taken into account. This indicates that the protonation of the complex also occurs under these circumstances,

although the $\lg K^{H}_{(AlNOTA)} = 1.9 \pm 0.3$ shows large standard deviation and it can be considered as just an upper limit. The ^{27}Al-NMR shift of the complex does not change with pH at our experimental conditions, pointing to a minor impact of protonation on the overall structure of the complex. The data points gained from ^{27}Al-NMR adhere more to distribution curves calculated without including a protonation constant (see Fig. 3), although the deviations are higher at lower pH. Stability and protonation constants of the complexes are defined by Eqs. 3 and 4:

$$K_{AlNOTA} = \frac{[Al(NOTA)]}{[Al^{3+}][NOTA^{3-}]} \tag{3}$$

$$K_{AlHNOTA} = \frac{[Al(HNOTA)^{+}]}{[Al(NOTA)][H^{+}]}. \tag{4}$$

The square brackets mean equilibrium concentrations. One can conclude that the formation constant of [Al(NOTA)], $\lg K_{[Al(NOTA)]} = 17.9(1)$, is quite high compared to that of [Gd(NOTA)] ($\lg K_{[Gd(NOTA)]} = 13.7$) [12]. The stability of [Al(EDTA)]$^{-}$, having 2 N and 4 O donor atoms is also smaller ($\lg K = 16.1$) compared to [Al(NOTA)] with 3 N and 3 O donors. This could be related to the macrocyclic effect and the size-match between the ligand cavity and the ionic radius: i.e. Al^{3+} fits very well into the cavity of the NOTA^{3-} ligand.

In order to inspect if the model based on the pH-potentiometric results was adequate, ^{27}Al-NMR signal intensities of Al(III) and [Al(NOTA)] measured at
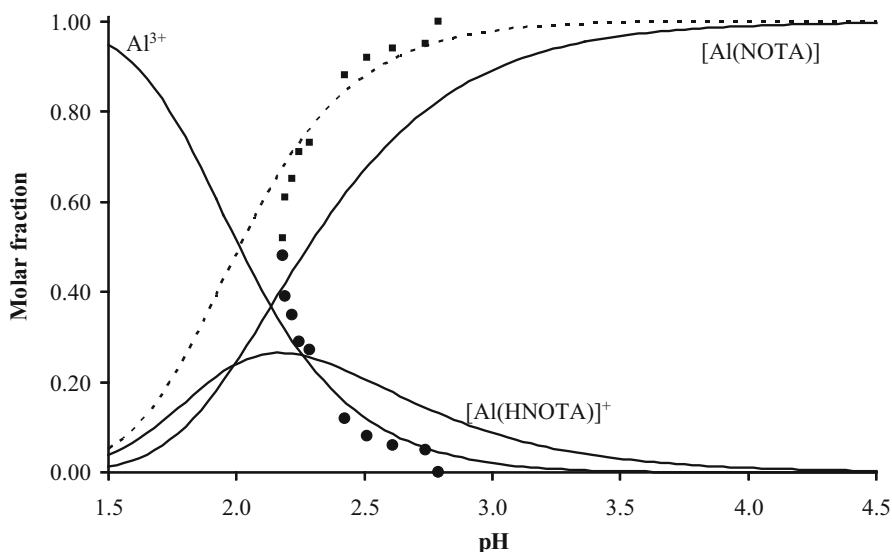

Fig. 3 Distribution curves of Al(III)-containing species in Al(III)-NOTA system ($c_{Al3+} = 3$ mM; $c_{NOTA} = 3.05$ mM). Lines correspond to pH-potentiometric results, the dashed line is the sum of fractions that contain NOTA-bound Al(III) species [Al(NOTA)] and [Al(HNOTA)]$^{+}$, data points obtained from ^{27}Al-NMR are marked with spots (free Al^{3+}) and squares (NOTA-bound Al(III)-species)

different pH values are shown on the Al(III)-distribution diagram. The results are shown in Fig. 3, along with calculated curves based on the pH-potentiometric data.

No new signal assignable to $[Al(HNOTA)]^+$ was observed due to protonation, the chemical shift is likely insensitive to ligand protonation. Altogether, the results seen in Fig. 3 strongly "validate" the model based on pH-potentiometry. The obtained protonation constant (see Table 1), although having a relatively large error, is similar to those usually observed for macrocyclic metal complexes [13–15].

Considering the formation of the mixed complex $[Al(EDTA)(OH)]^{2-}$ [16, 17] ($\lg K_{mix} = 5.9$), a titration was performed in the alkaline region in order to detect the mixed $[Al(NOTA)(OH)]^-$ complex. In fact, a proton release process was observed under basic conditions, which can be explained by the formation of the proposed ternary complex, as defined in Eqs. 5 and 6:

$$[Al(NOTA)] + H_2O \rightleftharpoons [Al(NOTA)(OH)]^- + H^+ \qquad (5)$$

$$K_{AlNOTA(OH)} = \frac{[Al(NOTA)(OH)^-][H^+]}{[Al(NOTA)]}. \qquad (6)$$

The value of $\lg K_{AlNOTA(OH)}$, determined to be $-12.2(1)$ is 6 orders of magnitude lower than the similar constant for $[Al(EDTA)(OH)]^{2-}$. Moreover, this ternary complex cannot be studied as a real equilibrium species since it slowly dissociates to $[Al(OH)_4]^-$ and $NOTA^{3-}$, although the titration curve could be described quite well with the above constant (in a "fast" titration: waiting time after addition of each dose of titrant NaOH was about 40 s). Considering the stability and protonation constants obtained for $[Al(NOTA)]$, as well as the hydrolysis constants of Al(III) ($\lg K_{11} = -5.48$; $\lg K_{12} = -9.9$; $\lg K_{13} = -15.6$; $\lg K_{14} = -23.0$) ion, distribution diagrams were calculated and plotted (Fig. 4).

In spite of the high thermodynamic stability of the $[Al(NOTA)]$ complex, the tetrahydroxido aluminate (III) complex is formed above pH 8. This is a slow process compared to the equilibration time applied during the pH-potentiometric titration, which explains why the distribution curves shown in the left side of Fig. 4 seem to be consistent with the "fast" titration data (real time titrations). If enough time is given for these system to equilibrate, the species found in the acidic region would be the same, while the speciation at pH>7.0 is expected to change
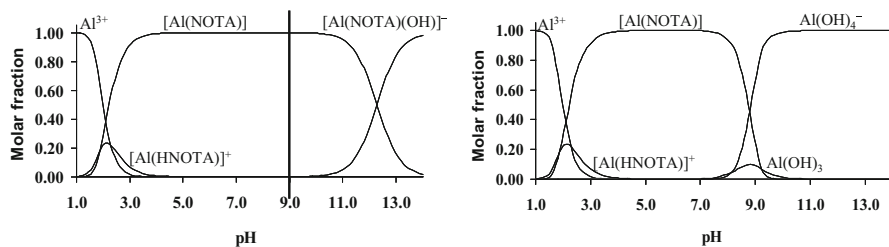

Fig. 4 Distribution of Al(III)-containing species in Al(III)-NOTA systems in a broad pH-range, made with- (*right*), and without (*left*) including Al(III) hydrolysis constants, $c_{Al} = 3$ mM; $c_{NOTA} = 3.05$ mM. The $[Al(NOTA)(OH)]^-$ complex is a metastable species (see text)

dramatically (as shown in the right part of Fig. 4). The transformation of the metastable system (shown left) to the equilibrium state (shown right), i.e. the dissociation kinetics of [Al(NOTA)] were further studied by ^{27}Al-NMR and are discussed in the next part of the paper.

Kinetic study

The high kinetic inertness of metal complexes used in living systems is one of the most important, if not the most important criterion for safe application. The dissociation of inert complexes is negligibly slow regardless of thermodynamic stability, which prevents in vivo release of (toxic) metal ions. For this reason, the kinetic properties of [Al(NOTA)] were investigated in detail. To the best of our knowledge, no such published data is available in the literature.

The in vivo dissociation of metal complexes (ML) can occur when there is either a competition reaction between the chelated metal ion and endogenous ones such as Zn^{2+} or Cu^{2+} for the ligand, or between the endogenous ligands for the metal ion in the complex. In other words, the presence of these competing components defines the thermodynamic condition set for the dissociation of ML. The dissociation mechanisms of metal complexes do not differ fundamentally from each other, the reaction most often proceeds via proton- and metal-assisted pathways. In the case of macrocyclic complexes, proton-assisted dissociation is generally the most effective path for dissociation, while metal ions and small endogenous anions (carbonate, citrate and phosphate) may sometimes play a role in accelerating the dissociation of ML complexes of open-chain ligands [18].

In our case, the kinetic inertness of [Al(NOTA)] was investigated both in acidic and basic conditions, at pH-values where the complex is thermodynamically unstable. In these cases, the kinetic inertness of [Al(NOTA)] can be characterized by the rate constants of H^+ and OH^- ion-catalyzed dissociation reactions, where protonated and ternary hydroxido complexes are formed as intermediates respectively (Eqs. 7 and 8):

$$[Al(NOTA)] + H^+ \rightleftharpoons [Al(HNOTA)]^+ \tag{7}$$

$$[Al(NOTA)] + OH^- \rightleftharpoons [Al(NOTA)(OH)]^- \tag{8}$$

The reactions were examined under pseudo-first order conditions, ensured by the high NaOH and H^+ concentration. The observed rate constants, k_{obs}, were fitted to Eq. 9, where k_{obs} is a pseudo-first order rate constant and $[Al(NOTA)]_t$ is the total concentration of the complex.
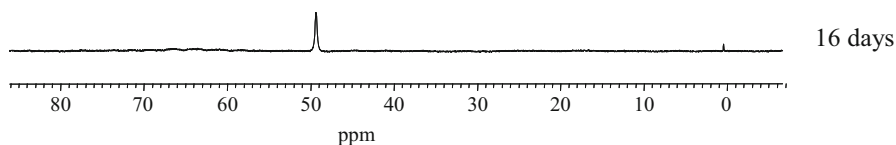

Fig. 5 ^{27}Al-NMR spectrum of 1 mM [Al(NOTA)] in 1 M HCl taken 16 days after the components were mixed

$$-\frac{d[Al(NOTA)]_t}{dt} = k_{obs}[Al(NOTA)]_t \qquad (9)$$

In order to obtain data for the acid-catalyzed dissociation, which was assumed to be quite slow, the reaction was investigated at high acid concentration. Fig. 5 only shows a very small signal (about 1.5 % of the overall intensity) of Al(III) at 0 ppm in 1.0 M HCl, even after 16 days, while the half-life of "gold standard" [Gd(DOTA)]⁻ was found to be a few hours in this medium [18] indicating an immeasurably slow rate of the acid catalyzed dissociation.

Usually, the first step of the proton-assisted dissociation of ML complexes formed with polyamino-polycarboxylate ligands, like NOTA, is the protonation of a pendant arm of the coordinated ligand. The proton is transferred in the second step to a nitrogen donor atom of the macrocyclic ring, followed by the dissociation of the complex. It seems that the compact structure of [Al(NOTA)] strongly hampers this proton transfer between the donor atoms, resulting in a very high inertness under acidic conditions.

Knowing the high affinity of Al(III) towards forming the tetrahydroxide [Al(OH)$_4$]⁻ complex, the dissociation of [Al(NOTA)] was also studied under basic conditions. Four NMR samples were prepared containing 1 mM of [Al(NOTA)] in 0.05–0.5 M NaOH, and their ^{27}Al-NMR spectra were recorded regularly during the next 3 days.
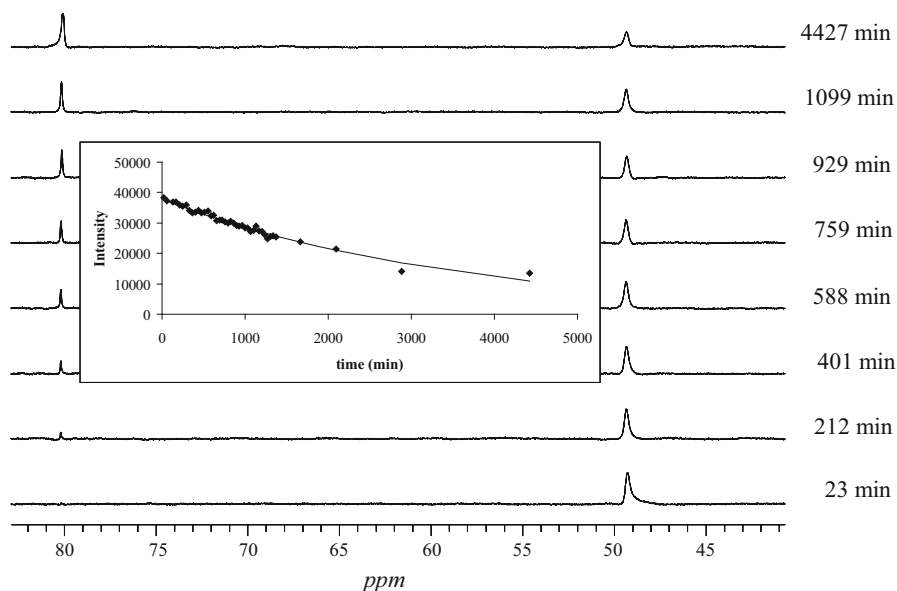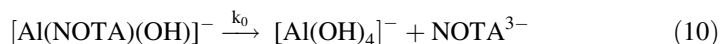

Fig. 6 Time-dependence of ^{27}Al-NMR spectra of 1 mM [Al(NOTA)] in 0.5 M NaOH, with the inset box indicating the decrease in [Al(NOTA)] signal intensity as a function of time

The dissociation reactions were followed by observing the changes in the signal intensity of [Al(NOTA)] over time (Fig. 6). As it is seen in Fig. 6, the dissociation of [Al(NOTA)] is relatively slow: the time required for equilibration in 0.5 M NaOH was more than 3 days shown by the decrease in the intensity of the [Al(NOTA)] signal at 48 ppm. The course of dissociation was followed with the signal intensity change of [Al(NOTA)].

Using the intensity/time data pairs, the first order rate constants were calculated for each NaOH concentration. According to the data presented in Fig. 7, reaction rates were found to be directly proportional to NaOH concentration.

Considering the result that [Al(NOTA)] is dominantly present as [Al(NOTA)(OH)]$^-$ ternary complex in this NaOH concentration range (mentioned earlier as an "intermediate"), the following reaction pathways can be assumed: [Al(NOTA)(OH)]$^-$ dissociates spontaneously (Eq. 10), indicated by the intercept of Fig. 7 and via a hydroxide-assisted reaction, which is characterized by the rate constant k_{OH} (Eq. 11):

$$[Al(NOTA)(OH)]^- \xrightarrow{k_0} [Al(OH)_4]^- + NOTA^{3-} \tag{10}$$

$$[Al(NOTA)(OH)]^- + OH^- \xrightarrow{k_{OH}} [Al(OH)_4]^- + NOTA^{3-}. \tag{11}$$

The rate law in alkaline medium can be expressed as follows:

$$k_{obs} = k_0 + k_1[OH^-] \tag{12}$$

Fig. 7 shows the best fit of the k_{obs} values to the Eq. 12. The values obtained for k_0 and k_1 are $(2.0 \pm 0.1) \times 10^{-6}\,s^{-1}$ and $(6.8 \pm 0.5) \times 10^{-6}\,M^{-1}s^{-1}$, respectively.

Because of the occurrence of various dissociation pathways characterizing the decomplexation of agents used in vivo, direct comparison of their kinetic inertness is difficult. Therefore, the half-lives ($t_{1/2}$) of dissociation under physiological conditions (pH = 7.4) are frequently used for comparison. The kinetic data obtained from our investigations show that decomplexation is accelerated by the formation of the ternary hydroxido complex. However, the proton-assisted
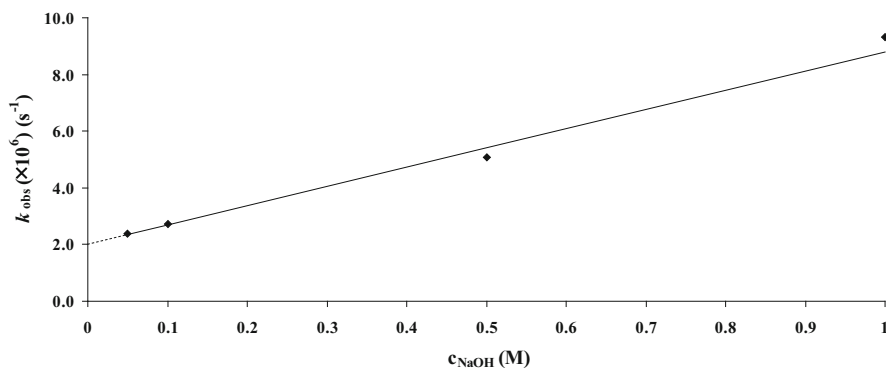

Fig. 7 Dependence of the pseudo first-order rate constants, k_{obs}, for the hydroxide-assisted dissociation of [Al(NOTA)] on the hydroxide concentration (25 °C, 1.0 M NaCl)

dissociation of [Al(NOTA)] does not play a significant role. Using the rate constants characterizing the hydroxide-assisted dissociation, the $t_{1/2}$ value of [Al(NOTA)] was calculated and found to be 94 h at physiological pH. Considering that the half-life of excretion is about 1.5 h for most contrast agents, the application of [Al(NOTA)] as a carrier for F^- ions may rightly be considered safe.

Preliminary results of ¹⁹F-NMR study of ternary complex formation

As it was indicated earlier [4, 5], [Al(NOTA)] is known to be capable of carrying the radioactive ¹⁸F-isotope that has a key role in PET investigations. Laverman and his coworkers developed a method for radiolabeling the Al(III) complex of an Octreotide derivative of NOTA, in which an acetate arm has been modified (amidated) to link the peptide [19]. This method uses high temperature (100 °C for 10–15 min) for the complexation in acetate buffer, since the formation rate of [Al(NOTA)] is relatively low under these conditions. The radiolabeled compound ([Al(¹⁸F)(NOTA)-octreotide]) was found to be intact in human serum after 4 h and no in vivo dissociation was detectable in mice after 30 min. In fact, this procedure provides a promising agent for PET investigations, but no information on the thermodynamic stability or kinetic inertness of the Al(III)-F-NOTA ternary complex, which, available so far, would be useful in predicting its long term in vivo behavior.

The radiolabeling experiments are performed at very low concentrations (at about 10^{-9} M). Therefore, the procedures cannot be directly adapted for mM concentrations used in ¹⁹F-NMR. However, we wanted to detect the formation of $[Al(NOTA)(F)]^-$ mixed complex both by fluoride selective electrode and by ¹⁹F-
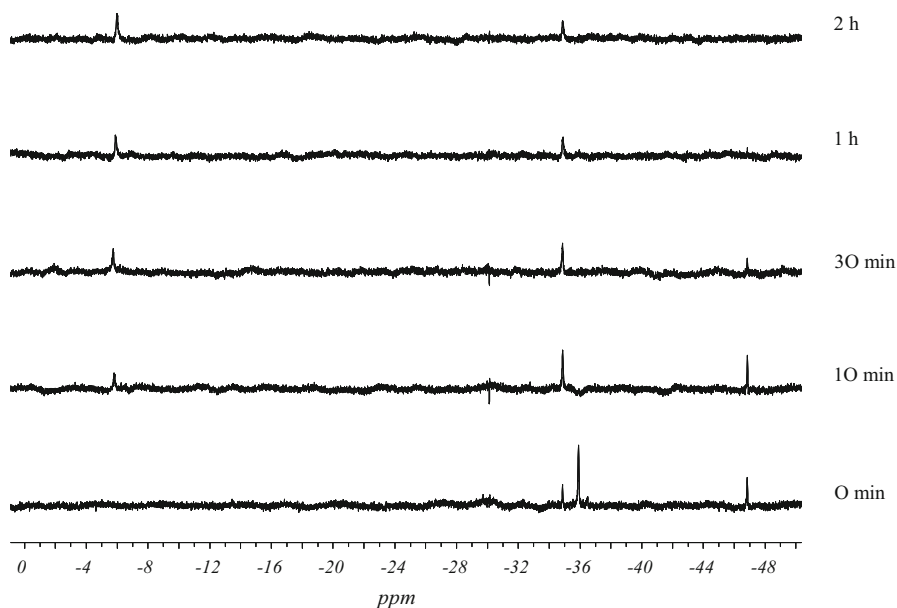

Fig. 8 376.5 MHz ¹⁹F-NMR spectra of samples containing Al(III), NOTA and F^- in equimolar amounts after heating in pH = 4 acetate buffer for varying times at 100 °C

NMR. Attempts were made to follow the formation of the mixed complex between [Al(NOTA)] and F^-, using both direct potentiometric titration and "out of cell" technique (see Supporting Information). In all cases, the potentials recorded by F^--selective electrode in both the presence and absence of (preprapared) [Al(NOTA)] complex were identical, indicating that the expected complex formation does not occur under these circumstances (25 °C, 0.15 M NaCl). An obvious explanation of this phenomenon can be the high rigidity of [Al(NOTA)]. Since the coordination sphere of the Al(III) ion is saturated with the donor atoms of NOTA, coordination of one further F^- ion to the inner sphere requires the dissociation of a carboxylic oxygen, which is an extremely slow process proven by the kinetic experiments in HCl. Therefore, in order to reach equilibrium, the samples were heated for 10 min at 100 °C as described in the literature [4], and their ^{19}F-NMR spectra were recorded after cooling to room-temperature. Only the signal of free F^- was detectable after this treatment, which means no detectable formation of the ternary complex, i.e. the formation is quite slow even at 100 °C. It should be noted, that the behavior of $[Al(EDTA)F]^{2-}$ could easily be followed by fluoride selective electrode. [20]

Fig. 8 shows the dependence of ^{19}F-NMR spectra in samples with equimolar Al(III), NOTA and fluoride on heating time, recorded 24 h afterwards. In the sample without heating, there is a new signal, most likely the signal of the ternary complex at −47 ppm among the signals of Al(III) fluorido complexes (−35 to 36 ppm) [21]. In the sample heated for 10 min, the signal of the free F^- ion appears and the signals of Al(III) fluorido complexes decrease, together with a slight increase in intensity of the mixed complex. Further treatment of the samples causes complete decomposition of the mixed complex to parent AlF_2^+ complex and F^- ion, shown by the signals at −35 ppm and −5 ppm, respectively. (The signal at −5 ppm is the time averaged signal of F^- and HF at pH 4.8 in acetate buffer.)

The formation of $[Al(NOTA)(F)]^-$ was almost 100 % in 50 % ethanol after 15 min of heating according to the ^{19}F-NMR spectrum (Fig. 9). The chemical shift was −48 ppm, slightly different from the −47 ppm measured in water likely due to solvent effect. The role of ethanol could be related to the decreased basicity of the ring nitrogen donor atoms in NOTA [22]; resulting in faster complex formation due to weaker competition between the H^+ ion and Al(III) for the donor atoms of the
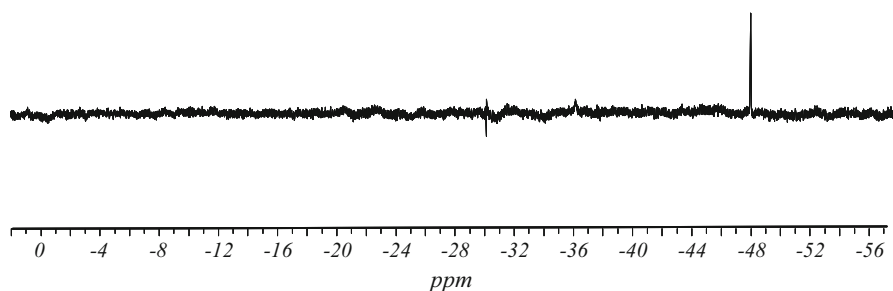

Fig. 9 376.5 MHz ^{19}F-NMR spectrum of a sample containing Al(III), NOTA and F^- in equimolar amounts after heating for 15 min at 100 °C in 50 % ethanol

macrocycle. A detailed study of formation kinetics for metal-macrocyclic complexes in mixed solvents are in progress in our laboratory.

Our preliminary results show that complex formation kinetics are fairly complicated in the Al(III)—NOTA^{3-}—H$^+$—F$^-$ four-component system, further experiments should be performed to describe and understand the formation kinetics of the mixed complex.

Conclusions

The formation kinetics of [Al(NOTA)] in acidic solutions is fairly slow. Therefore, equilibrium constants have been evaluated from pH-potentiometric data measured in "batch" samples aged for four months. ^{1}H- and ^{27}Al-NMR experiments were used to check the time course of equilibration and validate the equilibrium model consisting of [Al(NOTA)] and [HAl(NOTA)]$^+$ in acidic solution, with equilibrium constants lg$K_{Al(NOTA)}$ = 17.9(1) and lg$K^H_{Al(NOTA)}$ = 1.9(3). There is a metastable mixed hydroxido complex detected in alkaline solution, [Al(NOTA)(OH)]$^-$ with lg$K_{AlNOTA(OH)}$ = $-12.2(1)$.

The dissociation of [Al(NOTA)] in acidic solutions is immeasurably slow, only about 1.5 % of the complex is converted to Al(III) in 16 days in 1 M HCl. The dissociation in alkaline solution follows the rate law $k_{obs} = k_0 + k_1[OH^-]$ with $k_0 = (2.0 \pm 0.1) \times 10^{-6}\,s^{-1}$ and $k_1 = (6.8 \pm 0.5) \times 10^{-6}\,M^{-1}s^{-1}$. Using these kinetic parameters the half-life of [Al(NOTA)] dissociation was calculated to be $t_{1/2}$ = 94 h at physiological pH.

The formation of the ternary [Al(NOTA)(F)]$^-$ via direct reaction of [Al(NOTA)] and F$^-$ cannot be detected using fluoride selective electrode and/or ^{19}F-NMR spectroscopy. The kinetics is fairly complicated in the Al(III)—NOTA^{3-}—H$^+$—F$^-$ four component system. However a new signal in the ^{19}F-NMR spectrum at -48 ppm might be assigned to the ternary complex, which was formed in water–ethanol mixture with high yield.

References

1. Merbach AE, Helm L, Tóth É (eds) (2013) The chemistry of contrast agents in medical magnetic resonance imaging, 2nd edn. Wiley, New York
2. Clarke ET, Martell AE (1991) Inorg Chim Acta 181:273–328
3. D'Souza CA, McBride WJ, Sharkey RM, Todaro LJ, Goldenberg DM (2011) Bioconjugate Chem 22:1793–1803
4. Pan D, Yan Y, Yang R, Xu YP, Chen F, Wang L, Luo S, Yang M (2014) Contrast Media Mol Imaging 9:342–348

5. Dijkgraaf I, Terry SYA, McBride WJ, Goldenberg DM, Laverman P, Franssen GM, Oyen WJG, Boerman OC (2013) Contrast Media Mol Imaging 8:238–245
6. Kang J, Jo JH (2003) Bull Korean Chem Soc 24:1403–1406
7. Maheshwari V, Dearlin JLJ, Treves ST, Packard AB (2012) Inorg Chim Acta 393:318–323
8. Sajó I (1962) Komplexometria. Műszaki Könyvkiadó, Budapest p **118**
9. Irving HM, Miles MG, Pettit L (1967) Anal Chim Acta 38:475–488
10. Zékány L, Nagypál I (1985) In: Legett DJ, (ed) Computational method for determination of formation constants. Plenum New York p 291
11. Akinori J, Takashi K, Yuichi T, Shigeaki K (1990) Analytical Sciences 6:629–630
12. Brücher E, Cortes S, Chavez F, Sherry AD (1991) Inorg Chem 30:2092–2097
13. Försterová M, Svobodová I, Lubal P, Táborský P, Kotek J, Hermann P, Lukeš I (2007) Dalton Trans 535–549
14. Regueiro-Figueroa M, Bensenane B, Ruscsák E, Esteban-Gómez D, Charbonnière L J, Tircsó Gy, Tóth I, de Blas A, Rodríguez-Blas T, Platas-Iglesias C (2011) Inorg Chem 50:4125–4141
15. André JP, Brücher E, Király R, Carvalho RA, Mäcke H, Geraldes CFGC (2005) Helv Chim Acta 88:633–646
16. Schwarzenbach G, Gut R, Anderegg G (1954) Helv Chim Acta 37:937–957
17. Jószai R, Purgel M, Pápai I, Wakita H, Tóth I (2007) J Mol Liquids 131–132:72–80
18. Baranyai Z, Pálinkás Z, Uggeri F, Maiocchi A, Aime S, Brücher E (2012) Chem Eur J 18:16426–16435
19. Laverman P, McBride WJ, Sharkey RM, Eek A, Joosten L, Oyen WJG, Goldenberg DM, Boerman OC (2010) J Nucl Med 51:454–461
20. Nemes J, Tóth I, Zékány L (1998) J Chem Soc Dalton Trans 2707-2713
21. Bodor A, Tóth I, Bányai I, Szabó Z, Hefter CT (2000) Inorg Chem 39:530–2537
22. Rorabacher DB, MacKellar WJ, Shu FR, Sister MB (1971) Anal Chem 43:561–573