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NMR Determination of Free Gallium(III) Ions in Aqueous Solutions of Ga-complexes, “Cold” Analogs of PET/SPECT Tracers

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Abstract: Ga-complexes are widely used as radiopharmaceuticals for PET or SPECT imaging and as therapeutic agents. At physiological pH, free gallium(III) ions can stably exist as soluble gallate $[\text{Ga}(\text{OH})_4]^-$, a nephrotoxic compound whose presence should be avoided. Any Ga-complex, therefore, should be carefully checked for the absence of gallate before use. Here we show that ^{71}Ga NMR is a useful tool to rapidly detect the presence of gallate in aqueous solutions of Ga-complexes and to follow the purification steps of the Ga-complex solutions.

Keywords: Ga-complexes, $[\text{Ga}(\text{OH})_4]^-$ (gallate), ^{71}Ga NMR, PET, SPECT

1. Introduction

Ga-complexes are invaluable tools as probes for imaging studies with ^{66}Ga , ^{67}Ga , or ^{68}Ga radiopharmaceuticals, and as therapeutic agents for the treatment of a number of cancer and infectious diseases with non-radioactive gallium compounds (1). Their use relies on the assumption that the solutions are not contaminated by the presence of free Ga^{3+} ions. These free Ga^{3+} ions can have a profound effect on the efficacy of the gallium agents as they are likely to exist as $[\text{Ga}(\text{OH})_4]^-$ (gallate) (2) or to be taken up by iron-binding proteins (3). Large amounts of plasma gallate have been associated with nephrotoxicity (4-6). Therefore, any Ga-complex should be carefully checked for free Ga(III) ions before use. Various methods have already been proposed for the quantification of gallium ions in solution, such as spectrophotometry (7), fluorometry (8), complexometric titrations (9), or ICP-AES (10). As compared to the non-destructive NMR technique (11-12), those methods have the drawback that the measurement has to be carried out in acidic or strongly acidic media. In fact, most of the Ga-complexes undergo proton-assisted decomplexation in strongly acidic media. Herein, we report the use of ^{71}Ga NMR to rapidly detect the presence of free Ga(III) ions in the solutions of their complexes.

Gallium has two quadrupolar NMR active nuclei: ^{69}Ga and ^{71}Ga . In spite of a lower natural abundance, ^{71}Ga is generally the preferred isotope because of its higher

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3 sensitivity and lower quadrupolar moment ($0.112 \times 10^{-28} \text{ m}^2$ (13)). Compared with its
4 wide range of applications in solid-state NMR (14-15), ^{71}Ga NMR is less common
5 among the liquid NMR community probably due to the broad peaks generally
6 exhibited. A few articles have reported that the ^{71}Ga NMR spectra can be obtained
7 only when Ga(III) ions are in a highly symmetrical local environment such as in
8 octahedral $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$ or tetrahedral $[\text{Ga}(\text{OH})_4]^-$ (2, 11-12, 16), and C_3 -symmetric
9 Ga-NOTA derivatives (17-22). As a proof of concept, we have studied here a solution
10 of the approximately octahedral complex Ga-DOTA.
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14 2. Material and methods

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16 Gallium atomic absorption standard solution, gallium(III) nitrate hydrate, HNO_3 , HCl ,
17 NaOH , Chelex[®] 100 and D_2O were purchased from Sigma (Bornem, Belgium) and
18 used without further purification. Concentrations of gallium were measured by
19 ICP-AES analysis on a JOBIN YVON JY70⁺ spectrometer (Paris, France). Unpurified
20 Ga-DOTA was kindly provided by an industrial laboratory and its molecular weight
21 was determined to be 470 (ES-MS ($\text{M}+2\text{Na}$)⁺: 515.3, 517.3 (Ga isotopes)) from an
22 electrospray mass spectrum recorded on a Q-tof Ultima spectrometer (Micromass,
23 Manchester, UK). Distilled water was used throughout the experiments.
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27 The NMR spectra were recorded on an Avance 300 spectrometer (Bruker, Karlsruhe,
28 Germany) equipped with a 7.4 T superconducting magnet. The ^{71}Ga NMR spectra
29 were obtained with a 10 mm BBI probe at a frequency of 91.5 MHz. Spectra were
30 recorded using a spin-echo sequence ($\text{TR}=0.04 \text{ s}$, $\text{TE}=4 \mu\text{s}$), and were referenced to an
31 external solution prepared by diluting 126 μl of gallium atomic absorption standard
32 solution with a 0.34 M HNO_3 10% D_2O /90% H_2O solution (Total volume=360 μl ,
33 gallium(III) concentration=5 mM). The volume of the tested samples was kept
34 constant at 1800 μl . The temperature of the sample was fixed to 300 K by a Bruker
35 BVT-2000 unit.
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39 The removal of free gallium ions from the solution of Ga-DOTA was performed by
40 adding Chelex[®] 100 to a Ga-DOTA (approx. 27.45 mM, $\text{pH}=7.50$) 10% D_2O /90% H_2O
41 solution. The concentration of Chelex[®] 100 was fixed to 5% in weight. The mixture
42 was stirred during 24 h and centrifuged to collect the supernatant. This operation was
43 repeated once and the NMR spectrum of the supernatant, which pH is still 7.50, was
44 then recorded.
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48 3. Results and discussion

49 In aqueous solution and depending on the pH , the Ga(III) ion can form different kinds
50 of species, such as $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$, $[\text{Ga}(\text{OH})(\text{H}_2\text{O})_5]^{2+}$, $[\text{Ga}(\text{OH})_2(\text{H}_2\text{O})_4]^+$, $\text{Ga}(\text{OH})_3$,
51 $[\text{Ga}(\text{OH})_4]^-$, etc. (23-24). Among those Ga(III) ion species, only the octahedral
52 $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$ and the tetrahedral $[\text{Ga}(\text{OH})_4]^-$ can be observed by NMR, due to their
53 highly symmetrical local environment of the gallium nucleus (11-12) (Fig. 1).
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56 Under acidic conditions ($\text{pH} < 3.50$), the hexa-aqua ion, $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$, gives a peak
57 whose intensity gradually falls as the pH value increases indicating that the
58 $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$ species disappears from the solution while another one is forming. The
59 line broadening is due to the fast exchange between the octahedral $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$ and
60 the less symmetrical hydrolysis products. Under basic conditions, $[\text{Ga}(\text{OH})_4]^-$, can be

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observed around 222 ppm with respect to $[\text{Ga}(\text{H}_2\text{O})_6]^{3+}$ and its intensity raises gradually with the increase of pH value. In the pH range of 3.5-7.0, no gallium signal can be observed. Furthermore, the state of the solution also changes with the pH value: at low pH (pH < 3.5) and high pH (pH > 9.5), the solution is clear and transparent, whereas in the pH range 3.5-9.5, a white precipitate appears. Thus, around physiological pH, the species of free gallium ions which exist in solution are the insoluble amorphous $\text{Ga}(\text{OH})_3$ and the soluble $[\text{Ga}(\text{OH})_4]^-$. The presence of $\text{Ga}(\text{OH})_3$, as a white precipitate, can be easily observed and removed from the solution of Ga-complexes, while the presence of $[\text{Ga}(\text{OH})_4]^-$ is difficult to observe and is, therefore, often ignored. It has to be stressed that, in the context of the search for traces of free gallium contaminating solutions of gallium complexes, attention should be paid to avoid low pH (<5) and particularly high pH (>8) which induce dissociation of the complex, as nicely shown in ref.12 ($\tau_{1/2} = 12.2$ days and 6.2 hours for Ga-DOTA at pH = 0 and 10 respectively).

We collected the ^{71}Ga NMR spectrum of a Ga-DOTA solution which had not been treated for the removal of the free Ga(III) ions at a pH value of 7.50 (see the bottom spectrum of Fig. 2). Two absorptions were observed, a broad one around 137 ppm and a sharper one at 222 ppm corresponding to Ga-DOTA and free Ga(III) ions in the form of $[\text{Ga}(\text{OH})_4]^-$ respectively. These results show that ^{71}Ga NMR allows detecting the presence of free Ga(III) ions in a Ga-complex solution without destroying the Ga-complex if the spectrum is recorded at a pH ranging from 7.5 to 8 and over a period of time that is short with respect to the kinetics of a possible dissociation. In our case, the data collection was shorter than 2.5 hours guaranteeing no dissociation at pH 7.5. Assuming such a reasonable scanning time, the detection limit for free gallium, will be around 10 ppm on a room temperature probe at 7.4 T and 300 K.

After the synthesis of a Ga-complex, it is important to be able to remove any free ions. We tested the efficacy of Chelex[®] 100, which is a styrene-divinylbenzene co-polymer containing iminodiacetic acid groups and is well-known for its ability to bind metal ions (25-27). It has, for example, been successfully applied in removing the free gadolinium, lanthanum, and europium ions from their complex solutions (28). We performed ^{71}Ga NMR spectra of a Ga-DOTA solution before and after a treatment with 5% Chelex[®] 100 (Fig. 2). The results show the complete disappearance of the ^{71}Ga signal corresponding to the free Ga ions, whereas the signal of Ga-DOTA remains unchanged. This result confirms that Chelex[®] 100 is a suitable agent for the removal of free gallium ions from the solutions of Ga-complexes and illustrates the potential of ^{71}Ga NMR to follow the purification step of the Ga-complex solutions.

4. Conclusions

This work confirms that ^{71}Ga NMR is a useful tool to rapidly detect the presence and the removal of free gallium ions in aqueous solutions of Ga-complexes, provided that the pH be maintained between 7.5 and 8. Its extension to quantitative analysis with

the use of an external reference is straightforward and the detection limit in the ppm range can be reasonably expected.

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References

1. Bandoli G, Dolmella A, Tisato F, Porchia M, Refosco F. Mononuclear six-coordinated Ga(III) complexes: a comprehensive survey. *Coord Chem Rev* 2009; 253(1-2): 56-77.
2. Akitt JW, Kettle D. ^{71}Ga nuclear magnetic resonance investigation of aqueous gallium(III) and its hydrolysis. *Magn Reson Chem* 1989; 27(4): 377-379.
3. Aramini JM, McIntyre DD, Vogel HJ. Gallium(3+) binding to ovotransferrin and its half-molecules: a multinuclear NMR study. *J Am Chem Soc* 1994; 116(25): 11506-11511.
4. Newman RA, Brody AR, Krakoff IH. Gallium nitrate (NSC-15200) induced toxicity in the rat: a pharmacologic, histopathologic and microanalytical investigation. *Cancer* 1979; 44(5): 1728-1740.
5. Krakoff IH, Newman RA, Goldberg RS. Clinical toxicologic and pharmacologic studies of gallium nitrate. *Cancer* 1979; 44(5): 1722-1727.
6. Bernstein LR, Tanner T, Godfrey C, Noll B, Chemistry and pharmacokinetics of gallium maltolate, a compound with high oral gallium bioavailability. *Met Based Drugs* 2000; 7(1): 33-47.
7. Onishi H. Detection of gallium with rhodamine B. *Anal Chem* 1955; 27(5): 832.
8. Urena E, Garcia de Torres A, Cano Pavon JM, Gomez Ariza JL. Determination of traces of gallium in biological materials by fluorometry. *Anal Chem* 1985; 57(12): 2309-2311.
9. Elsheimer HN. Complexometric determination of gallium with calcein blue as indicator. *Talanta* 1967; 14(1): 97-102.
10. Ouddane B, Skiker M, Fischer JC, Wartel M. Determination of trace metals in Estuarine Waters by ICP-AES after Ga-Mg coprecipitation. *Analisis* 1997; 25(9-10): 308-313.
11. Garcia R, Fouskova P, Gano L, Paulo A, Campello P, Toth E, Santos I. A quinazoline-derivative DOTA-type gallium(III) complex for targeting epidermal growth factor receptors: synthesis, characterisation and biological studies. *J Biol Inorg Chem* 2009; 14(2): 261-271.
12. Kubicek V, Havlickova J, Kotek J, Tircso G, Hermann P, Toth E, Lukes I. Gallium(III) complexes of DOTA and DOTA-monoamide: kinetic and thermodynamic studies. *Inorg Chem* 2010; 49: 10960-10969.

13. Andre JP, Macke HR. NMR spectroscopy of group 13 metal ions: biologically relevant aspects. *J Inorg Biochem* 2003; 97(4): 315-323.
14. Timken HKC, Oldfield E. Solid-state gallium-69 and gallium-71 nuclear magnetic resonance spectroscopic studies of gallium analogue zeolites and related systems. *J Am Chem Soc* 1987; 109(25): 7669-7673.
15. Turcu F, Simon S, Constantinescu S, Grecu N, Iuga D. Structural changes of piezoelectric $\text{La}_3\text{Ga}_5\text{SiO}_{14}$ induced by paramagnetic ions revealed by ^{71}Ga multiple quantum magic angle spinning. *Solid State Nucl Magn Reson* 2009; 36(2): 92-95.
16. Glickson JD, Pitner TP, Webb J, Gams RA. Hydrogen-1 and gallium-71 nuclear magnetic resonance study of gallium citrate in aqueous solution. *J Am Chem Soc* 1975; 97(7): 1679-1683.
17. Craig AS, Parker D, Adams H, Bailey NA, Stability, ^{71}Ga NMR, and crystal structure of a neutral gallium(III) complex of 1,4,7-triazaclononetriacetate: a potential radiopharmaceutical ?. *J Chem Soc, Chem Commun* 1989; (23): 1793-1794.
18. Moore DA, Fanwick PE, Welch MJ, A novel hexachelating amino-thiol ligand and its complex with gallium(III). *Inorg Chem* 1990; 29(4): 672-676.
19. Broan CJ, Jankowski KJ, Katakya R, Parker D, Synthesis and complex stability of parent and C-functionalised derivatives of 1,4,7-triazacyclononane-1,4,7-tris[methylene(methylphosphinic acid)]: an effective new complexing agent. *J Chem Soc, Chem Commun* 1990; (23): 1738-1739.
20. Broan CJ, Cox JPL, Craig AS, Katakya R, Parker D, Harrison A, Randall AM, Ferguson G, Structure and solution stability of indium and gallium complexes of 1,4,7-triazacyclononanetriacetate and of yttrium complexes of 1,4,7,10-tetraazacyclododecanetetraacetate and related ligands: kinetically stable complexes for use in imaging and radioimmunotherapy. X-Ray molecular structure of the indium and gallium complexes of 1,4,7-triazacyclononane-1,4,7-triacetic acid. *J Chem Soc, Perkin Trans 2* 1991; (1): 87-99.
21. Andre JP, Maecke HR, Zehnder M, Macko L, Akyel KG, 1,4,7-Triazacyclononane-1-succinic acid-4,7-diacetic acid (NODASA): a new bifunctional chelator for radio gallium-labelling of biomolecules. *Chem Commun* 1998; (12): 1301-1302.
22. Prata MIM, Santos AC, Geraldies CFGC, de Lima JJP, Structural and in vivo studies of metal chelates of Ga(III) relevant to biomedical imaging. *J Inorg Biochem* 2000; 79(1-4): 359-363.
23. Bernstein LR. Mechanisms of therapeutic activity for gallium. *Pharmacol Rev* 1998; 50(4): 665-682.
24. Reichert DE, Lewis JS, Anderson CJ. Metal complexes as diagnostic tools. *Coord Chem Rev* 1999; 184(1): 3-66.
25. Koshima H. Adsorption of iron(III), gold(III), gallium(III), thallium(III) and antimony(V) on Amberlite XAD and Chelex 100 resins from hydrochloric acid solution. *Anal Sci* 1986; 2(3): 255-260.

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26. Liu Y, Ingle JD, Jr. Automated two-column ion exchange system for determination of the speciation of trace metals in natural waters. *Anal Chem* 1989; 61(6): 525-529.
27. Kingston HM, Greenberg RR. An elemental rationing technique for assessing concentration data from a complex water system. *Environ Int* 1984; 10(2): 153-161.
28. Henoumont C, Henrotte V, Laurent S, Vander Elst L, Muller RN. Synthesis of a new gadolinium complex with a high affinity for human serum albumin and its manifold physicochemical characterization by proton relaxation rate analysis, NMR diffusometry and electrospray mass spectrometry. *J Inorg Biochem* 2008; 102(4): 721-730.

Captions:

Figure 1: ^{71}Ga NMR spectra of 10 mM gallium(III) nitrate hydrate (TR=0.04 s, TE=4 μs , 20 k scans). From bottom to top, the pH of the solution is 0, 3.0, 7.0, 7.5, 10.0 and 14.0, respectively.

Figure 2: ^{71}Ga NMR spectra of the aqueous solution of Ga-DOTA (approx. 27.45 mM, pH=7.50) (*: reference, 120 k scans, TR=0.04 s, TE= 4 μs). The bottom spectrum corresponds to the solution which has not been treated by Chelex[®] 100, whereas the top spectrum corresponds to the treated solution.

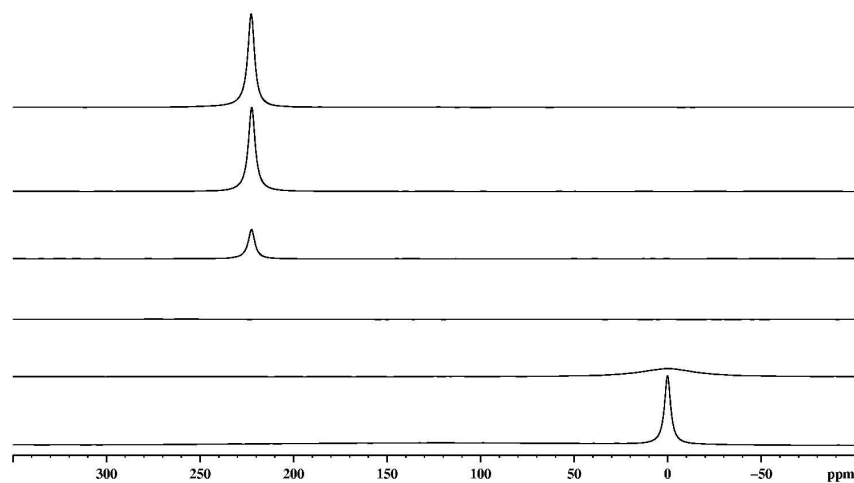


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297x209mm (300 x 300 DPI)

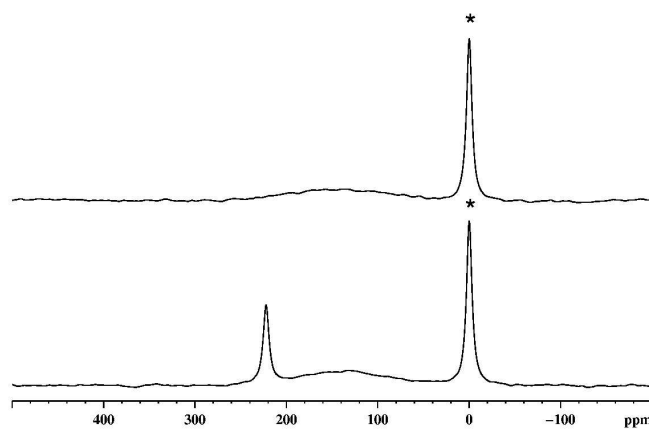


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