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ORIGINAL RESEARCH ARTICLE

High rigid Gd (DO3VA) shows remarkable relaxivity: A novel class of MMI agent engineered for MR analysis

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ABSTRACT

A novel dinuclear gadolinium(III) complex of an amide linked bispolyazatricarboxylate macrocycle (DO3VA) having 2-bromoisovaleric acid pendant arm is reported. The molecular longitudinal relaxivity of the dinuclear complex $[Gd_2\{acamidoet(DO3VA)_2\}(H_2O)_2]$ is 13.23$mM^{-1}s^{-1}$ which corresponds to a "per Gd" relaxivity of 6.62 mM^{-1} s^{-1} (20 MHz, 37 ± 0.1 °C, pH 7). The "per Gd" r1p value is higher than the relaxivity of the clinically approved CAs. The transverse relaxivity (r_{2p}) of $[Gd_2\{acamidoet(DO3VA)_2\}(H_2O)_2]$ is 14.34 mM^{-1} s^{-1}. The r_{2p}/r_{1p} values of 1.08 indicate that the complex is T_1-weighted CAs. The 2-bromoisovaleric acid seems to be an excellent pendant arm for holding Gd(III) metal ion at any pH. The remarkable stability of the complex at various pH and in presence of protein shows that the ligand can be used as functionality in making new CAs for MRI and the amide core is a versatile core molecule for the creation of polynuclear gadolinium(III) chelates and dendrimeric CAs.

Keywords: MRA CAs; Dinuclear Gd(III) Complex; 2-Bomoisovaleric Acid; pH and HSA Responsive CAs

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1. Introduction

Magnetic resonance imaging (MRI) is a powerful diagnostic modality because it provides non-invasive 3D visualization of anatomy within an arbitrary plane with superb soft tissue contrast. MRI is also a powerful analytical modality because it enables investigation of vascular and tissue physiology and pathology using quantitative biomarkers[1–4]. Due to high spatial resolution[5], MRI has become the clinical imaging procedure for a large number of studies involving the central nervous system, especially the brain[6], visualizing soft tissues[7], diagnosis of a variety of diseases and the assessment of almost all organs[8], and imaging of physiological properties such as diffusion, perfusion, and vascularity of tissues[5,9–12]. MRI also provides real-time images of visualizing drug delivery[13], monitoring biological processes, and following functional changes in vivo[14–16]. The image distinguish obtain in MRI is a three-dimensional signal intensity map of in-vivo water molecules. Difference in MRI images arises from difference in the relaxation times among water protons caused by differences in the neighboring background in tissues[17]. For many clinical applications, it is a common practice to administer an exogenous chemical, called contrast enhancing agent, to improve the image contrast[18]. Currently, about 30% of the MRI examinations use contrast agents[7,19–21].

The relaxivity reflect the competence of a contrast agent to accelerate longitudinal and transverse water proton relaxation rates $1/T_1$ and $1/T_2$, respectively, normalized to one millimole concentration of the agent[17,22]. The indication practical in MRI tends to augment with an boost in $1/T_1$ and diminish with an raise in $1/T_2$ and it is typical for contrast agents to influence both $1/T_1$ and $1/T_2$ to unreliable degrees[17]. Positive contrast agents increase the brightness of images while the negative contrast agents give dark images. Superparamagnetic materials like iron oxide nanoparticles are engaged as negative CAs[1–3]. Low molecular weight complexes of paramagnetic metal ions are administered as contrast agents prior to the acquisition of MRI images[1–3]. Due to the large magnetic moment ($\mu_{eff}^2 = g^2(S(S+1)) = 63$) by a long electron spin relaxation time (10^{-9} s). Gd(III) is extensively used as a contrast agent for MRI[23]. Free gadolinium ions get accumulated as phosphate salts in lysomes of heptocytes and macrophages in spleen, bone marrow, and lungs and as a result of its high in vivo toxicity Gd(III) ion is complexed with ligands to form stable complexes[24,25]. The commercially used contrast agents are Gd(III) complexes of polyaminocarboxylate ligands.

A substantial enhance in relaxivity is achieve if Gd(III) chelates are covalently or non- covalently associated to macromolecules[26]. The incorporation of gadolinium(III) chelates to polymers, dendrimers, or biological molecules increases the rotational correlation time and improve the relaxivity “per Gd” atom. Targeting polynuclear conjugates are envision to make available MRI with the talent to image the low-concentration receptors by delivering a large payload of Gd(III) chelates. The present investigation could also be extended to the development of macromolecular CAs by appending the Gd(DO3VA) chelate onto the macromolecular substrates such as polymers[27], proteins, and dendrimers[28].

In our previous work, we have reported two dinuclear contrast agents with enhanced relaxivities compared to the mononuclear agents[29–32]. The mononuclear Gd(III) complexes of 2-isovaleric acid functionalized cyclen derivatives and polynuclear Gd(DO3VA) complexes covalently conjugated to different amide linkers and core molecules can be subjected to detailed kinetic and pharmacokinetic studies to evaluate their suitability as contrast agents. Additional relaxivity gains may be achieved by increasing the number of the mobile protons of the amide linkers by the prototropic mechanism. The incorporation of 2-isovaleric acid groups is expected to increase the molecular weight as well as molecular dimension of the complex resulting in a longer rotational correlation time and thus leading to high proton relaxivity. The relaxivity of the mononuclear and polynuclear Gd(III) chelates of DO3VA depends on the residence time of the inner-sphere water molecules and the steric crowding and the formation of a secondary hydration sphere by the bulky 2-isovaleric acid group. Thus, there is a wide scope of studying the rotational dynamics and other relaxivity parameters for these systems. The gains made in the relaxivity of this mono- and polynuclear gadolinium complexes would shed more light on the design parameters for future MRI contrast agents.

After the first generation clinically approved extracellular contrast agents, magnetic resonance angiography represent a further striking practice for doctors in vascular imaging. The addition of lipophilic groups to the carbon backbone of polyazapoly carboxylates profoundly alters the pharma cokinetic and biodistribution properties of their gadolinium complexes. Furthermore, the interaction of the Gd(-DO3VA) complexes with human serum albumin (HSA), their stability at the physiological pH, and their vascular retention time can be studied to evaluate their suitability as contrast agents for magnetic resonance angiography (MRA).

2. Experimental section

2.1 Materials

Bis(3-aminopropylamine), benzene- sulfonyl chloride, TRIS base, Celite®, xylenol orange, and molecular sieves (4Å) (Fluka); 2-bromoisovaleric acid and gadolinium(III) chloride (Aldrich); cyclen (Strem chemicals); and 2-chloroacetyl chloride, sodium bromide, potassium hydroxide, sodium hydroxide, disodium hydrogen phosphate, magnesium turnings, iodine, and sodium carbonate (anhydrous) (Merck, India) were used as such. Charcoal (Merck, India) was used for the purification of compounds after activating by heating at 150 °C overnight. Calcium chloride (anhydrous) and sodium wire (Merck, India) were used for drying

purposes. Hydrochloric acid (AR, 35.4%, d = 1.18, Merck India) was used as supplied. Silica gel (blue indicator, Fluka) was used as desiccant after activating by heating in an oven at 200 °C overnight. Amberlite IR-120 (H^+ form, 16-45 mesh), Amberlite IR-400 (Cl^- form, 20-50 mesh), Dowex 50W x 8-200 (H^+-form, 8% cross linking, 100-200 mesh), and Dowex 1 x 8-400 (Cl^- form, 8% cross linking, 200-400 mesh) (Aldrich) were washed with double distilled water five times before use. KBr (FT-IR grade), DMSO-d_6 (99.9 atom % D), acetone-d_6 (99.9 atom % D), D_2O (100 atom % D) (Aldrich), and mineral oil (for IR spectroscopy, Fluka) were used as received. Super dry ethanol, super dry methanol, and triply distilled water were prepared by the standard procedures[33]. HPLC grade water (Merck, India) was used to prepare solutions of the complexes for relaxivity measurements.

3. Methods

3.1 Physical measurements

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR Spectrometer in the series of 4000-400 cm^{-1}. The electrospray ionization mass spectra were recorded on a micromass Quattro II triple quadrupole mass spectrometer. CHN microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin-Elmer AD 6 Autobalance. Helium was used as the carrier gas. ^1H and ^{13}C NMR spectra were recorded on a **Bruker AVANCE III 500 MHz multinuclei NMR** spectrometer. Broad band gradient probe head 'BBO' 5 mm probe was used for the ^1H NMR measurements. **Inverse Quad Probe head 5 mm 'QXI'** was used for the ^{13}C NMR measurements. 400 MHz ^1H NMR spectra were recorded on a Jeol GSX-400 multinuclear NMR spectrometer working at 25 ºC. The 100 MHz ^{13}C NMR spectra were recorded using Jeol GSX-400 instrument. pH measurements were made using Labindia PICO$^+$ pH/Conductive meter (3P) calibrated with buffer solutions of pH 4.0, 7.0, and 9.2[34].

3.2 Longitudinal relaxivity measurements

The T_1 measurements were performed on a Bruker minispec mq 20 NMR Analyzer operating at a frequency of 20 MHz and the temperature was maintained using a temperature console at 37 ± 0.1 °C. The solutions of the complexes were taken in a 180 x 10 mm stoppered glass tube. The instrument parameters were optimized for each T_1 measurement[24]. The computer program "Excel" (version 2013) was used to plot graph. The instrument was standardized by recording the relaxivity of FDA approved [Gd(DO3A)$(H_2O)_2$] in water (r_{1p} = 4.63 mM^{-1} s^{-1}, 20 MHz and 37 °C; lit.[34], 4.80 mM^{-1} s^{-1}, 20 MHz, 40 °C).

3.3 Transverse relaxivity measurements

The transverse relaxation time (T_2) was calculated by the standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (90°-τ-180°) with a τ value of 50 μs[35]. All other experimental conditions are the same as that employed for the T_1 measurements.

4. Synthesis of ligands and complexes

4.1 1,2-bis(2-chloroacetamido)ethane (1)

The *N*-alkylation of 1,2-diaminoethane (1 equiv) with 2-chloroacetyl chloride (2 equiv) in the presence of sodium carbonate in dry acetonitrile gives 1 as a pale yellow hygroscopic solid in 83% yield[36], yield (5.30 g, 83%), mp 180 °C. Anal. calcd. for $C_6H_{10}N_2O_2Cl_2$ (M_r = 213): C, 33.79; H, 4.73; N, 12.95. Found: C, 33.75; H, 4.68; N, 12.96. IR (KBr, cm^{-1}): 3304 ν(N-H) (*amide*), 2860 ν(C-H), 1657 ν(C=O) (*amide*), 1542 δ(N-H) (*amide*), 1406 ν(C-N), 546 ν(C-Cl). MS (ESI): *m/z* 235 $[(M-1)+Na]^+$, 173 $[(M-4)-Cl]^+$[37,38].

4.2 {1,4,7,10-tetraazacyclododecane-4,7,10-tris-2-iso-valeric acid} DO3VA (L1)

A solution of 2-bromoisovaleric acid (6.31 g, 34.86 mmol) in 50 mL water was slowly added to a solution of cyclen (2 g, 11.62 mmol) and sodium hydroxide (1.39 g, 34.86 mmol) in 150 mL water in a double surface round bottom flask, connected to a cryogenic water circulator bath and placed over a magnetic stirrer. The pH of the solution was maintained at 10 by adding 1 N aqueous sodium hydroxide solution for 12 h. It was cooled to room temperature, filtered, and dried in vacuum. The desired tri *N*-alkylated product DO3VA[23] was separated from the tetra *N*-alkylated product by ion exchange column chromatography (Amberlite, H^+ form) by eluting with water. Colorless crystalline solid, yield (3.96

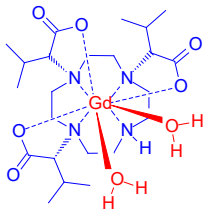
g) 72%, mp 160 °C. Anal. calcd. for $C_{23}H_{44}N_4O_6$ (M_r = 473): C, 58.45; H, 9.38; N, 11.85. Found: C, 58.43; H, 9.36; N, 11.81. ESI MS: *m/z* 473 $[M]^+$, 411 $[M\text{-}CH_2O_3]^+$, 379 $[(M\text{-}4)\text{-}C_2H_4O_4]^+$, 275 $[(M+4)\text{-}C_{10}H_{18}O_4]^+$. ^{1}H NMR ($D_2O$, 278 K): δ 1.29 (9 H, d, $^aCH_3$), 1.40 (9 H, d, $^bCH_3$), 2.50 (3 H, m, CH_3-^cCH-CH_3), 3.48 (16 H, t, $^dCH_2$), 4.41 (3 H, d, ^eCH). ^{13}C NMR (D_2O, 278 K): δ 15.8 ($^aCH_3$), 18.7 ($^bCH_3$), 28.1 (^cCH), 31.5 ($^dCH_2$), 49.5 ($^eCH_2$), 52.6 ($^fCH_2$), 77.3 ($^gCH_2$), 181.1 (COOH).

4.3 1,2-bis[1-(4,7,10-tris-2-isovaleric acid-1,4,7,10-tetraazacyclododecanyl)acetamido] ethane [aca midoet (DO3VA)$_2$, L2

A solution of DO3VA (L1) (2.83 g, 6 mmol) in water (50 mL) was added dropwise to a suspension of sodium carbonate (anhydrous) (0.64 g, 6 mmol) and 1,2-bis(chloroacetamido)ethane (1) (0.64 g, 3 mmol) in 100 mL of water at 60 °C over a period of 30 min. The resulting white hygroscopic solid was washed with dry methanol and recrystallized in water: yield (2.77 g, 85%), mp > 200 °C (dec). Anal. calcd. for $C_{52}H_{96}N_{10}O_{14}$ (M_r = 1085): C, 57.54; H, 8.91; N, 12.85. Found: C, 57.58; H, 8.92; N, 12.84. IR (KBr, cm^{-1}): 3486 ν(O-H) (*acid*), 3139 ν(N-H), 2965 ν(C-H), 2868 ν(C-H), 1609 ν(C=O) (*acid*), 1257 δ(N-H) (*amide*), 752 ω(N-H), 543 ρ(CH_3). MS (ESI): m/z 1082 $[M\text{-}3]^+$, 1013 $[(M\text{-}4)\text{-}4OH]^+$.

4.4 [Gd(DO3VA)(H$_2$O)$_2$] (I)

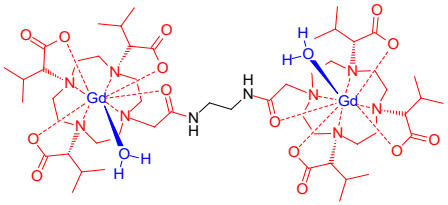
A solution of the ligand L1 (2.37 g, 5 mmol) in 30 mL of water was added to a solution of $GdCl_3.6H_2O$ (1.85 g, 5 mmol) in 30 mL of water. The pH of the solution was maintained at 7 throughout the reaction by adding an aqueous solution of NaOH and heated to 60 °C under argon atmosphere for about 12 h. It was cooled to room temperature, filtered through a 0.2 µm filter funnel, flash evaporated, and dried in vacuum. The resulting colorless hygroscopic solid was purified by column chromatography (Dowex ion exchange resin, cationic) by eluting with water and recrystallized in water: yield (2.82 g, 85%). Anal. calcd. for $C_{23}H_{45}N_4O_8Gd$ (M_r = 663): C, 41.62; H, 6.84; N, 8.45. Found: C, 41.59; H, 6.81; N, 8.46. IR (KBr, cm^{-1}): 3440 ν(O-H), 3138 ν(N-H) (*secondary amine*), 2965 ν(C-H), 1626 ν(C=O) (*acid*), 1048 ν(C-N) (*tertiary amine*), 728 ρ(CH_3) (*isopropyl group*), 629 ρ(O-H), 549 ω(O-H), 449 ν(Gd-O). MS (ESI): *m/z* 699 $[(M\text{-}3)+K]^+$, 443 $[(M+3)\text{-}GdC_2H_{10}O_2]^+$.



[Gd(DO3VA)(H$_2$O)$_2$] (I)

4.5 [Gd$_2${acamidoet(DO3VA)$_2$}(H$_2$O)$_2$] (II)

To a solution of the ligand L2 (5.42 g, 5 mmol) in 30 mL of water was added to a solution of $GdCl_3.6H_2O$ (3.72 g, 10 mmol) in 30 mL of water. The pH of the reaction mixture was maintained at 7 overnight by adding an aqueous solution of NaOH and heated to 60 °C under argon atmosphere for about 15 h. It was cooled to room temperature, filtered through a 0.2 µm filter funnel, flash evaporated, and dried in vacuum. The resulting colorless hygroscopic solid was purified by column chromatography (Dowex ion exchange resin, cationic) by eluting with water and recrystallized in water: yield (5.6 g, 78%). Anal. calcd. for $C_{52}H_{94}N_{10}O_{16}Gd_2$ (M_r = 1436): C, 43.68; H, 6.63; N, 9.80. Found: C, 43.72; H, 6.59; N, 9.79. IR (KBr, cm^{-1}): 3439 ν(O-H), 2965 ν(C-H), 1627 ν(C=O) (*acid*), 1256 δ(N-H) (*amide*), 1048 ν(C-N) (*tertiary amine*), 738 ρ(CH_3) (*isopropyl group*), 674 ρ(O-H), 549 ω(O-H), 448 ν(Gd-O). MS (ESI): m/z 1436 $[M]^+$, 1259 $[(M\text{-}2)\text{-}GdH_2O]^+$, 844 $[(M+3)\text{-}C_{11}H_{20}O_8Gd_2]^+$.



[Gd$_2${acamidoet(DO3VA)$_2$}(H$_2$O)$_2$]

5. Relaxivity studies

5.1 Longitudinal relaxivity of [Gd(DO3VA)(H$_2$O)$_2$] (I)

The complex exhibits longitudinal relaxivity of 5.32 $mM^{-1}s^{-1}$ (20 MHz, 37 ± 0.1 °C, pH 7) (**Figure 1**) which is higher than that of the clinically approved CAs. The higher relaxivity is due to the presence of

two inner-sphere water molecules (q = 2) and to a marginal increase in the molecular dimension and weight of the complex. The complex is expected to have the same coordination sphere as that of the acetic acid analogue [Gd(DO3A)(H_2O)$_2$]. Therefore, the higher relaxivity of [Gd(DO3VA)(H_2O)$_2$] compared to that of [Gd(DO3A)(H_2O)$_2$] is attributed to the rigid nature of the 2-isovaleric acid pendant arms which increase the relaxivity of the gadolinium(III) chelate by 10.83%.

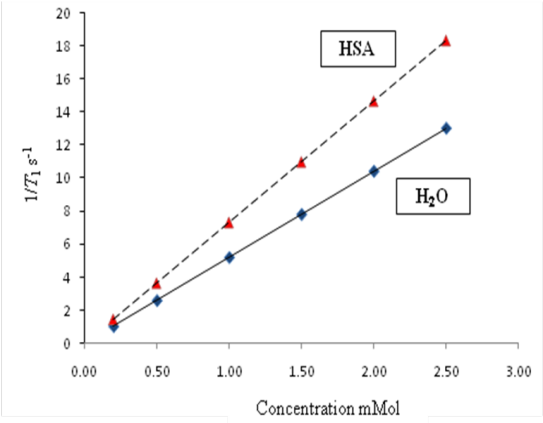


Figure 1. Longitudinal relaxivity of the complex (I) in water and HSA.

5.2 Longitudinal relaxivity of [Gd$_2${acamido-et (DO3VA)$_2$}(H_2O)$_2$] (II)

The complex exhibits longitudinal relaxivity of 13.25 mM^{-1} s^{-1} which corresponds to 6.62 "per Gd", a value higher than for other systems with q = 2. The high r_{1p} value of the complex is due to the presence of two inner-sphere water molecules coordinated to each Gd(III) ion (**Figure 2**). The complex [Gd(DO3A)(H_2O)$_2$] exhibits a longitudinal relaxivity of 4.8 mM^{-1} s^{-1} at 20 MHz and the higher relaxivity of [Gd$_2${acamido-et (DO3VA)$_2$} (H_2O)$_2$] indicates that the amide spacer which links two [Gd(DO3VA)] does not compromise the relaxivity of the complex. The enhancement of longitudinal relaxivity is a consequence of an increase in the molecular dimension which increases the rotational correlation time, τ_R[1,2,7,9]. The ligand acamidoet-(DO3VA)$_2$ contains two DO3VA unites linked by the rigid acetamido- ethane linker. The linker contains two mobile hydrogen atoms which may also contribute to the overall relaxivity enhancement through prototropic mechanism.

5.3 Transverse relaxivity

The transverse relaxivity of the complex [Gd(-

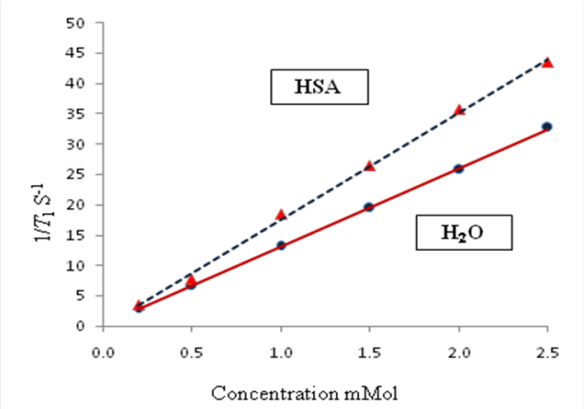


Figure 2. Longitudinal relaxivity of the complex (II) in water and HSA.

DO3VA)(H_2O)$_2$] is 7.69 mM^{-1} s^{-1} which is higher than that of other mononuclear gadolinium(III) complexes (**Figure 3**). The higher r_{2p} values for the complex is due to the high local concentration of [Gd(DO-3VA) (H_2O)] surface functionalities in a single molecule[23]. The r_{2p}/r_{1p} values for the mono- nuclear complex is 1.45, which indicate the complex is T_1-weighted MRI CAs.

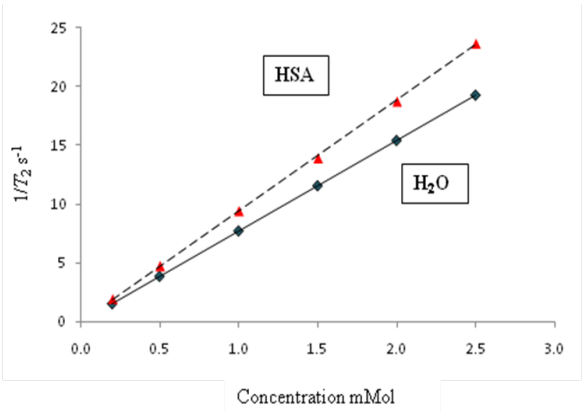


Figure 3. Transverse relaxivity of the complex (I) in water and HSA.

5.4 Transverse relaxivity of [Gd$_2${acamido-et (DO3VA)$_2$}(H_2O)$_2$] (L2)

The transverse relaxivity of [Gd$_2${acamido-et(-DO3VA)$_2$} (H_2O)$_2$] is 14.34 mM^{-1} s^{-1}. This higher r_{2p} value is due to the presence of large number of hydrogen bonded water molecules on the periphery of the 2-isovaleric acid pendant arms (**Figure 4**). The presence of two amide groups in the linker increases the rigidity of the complex[39,40] and the presence of two replaceable hydrogen atoms may enhance the overall relaxivity through the prototropic mechanism. The r_{2p}/r_{1p} value for the complex is 1.08 showing that it is a T_1-weighted contrast agent.

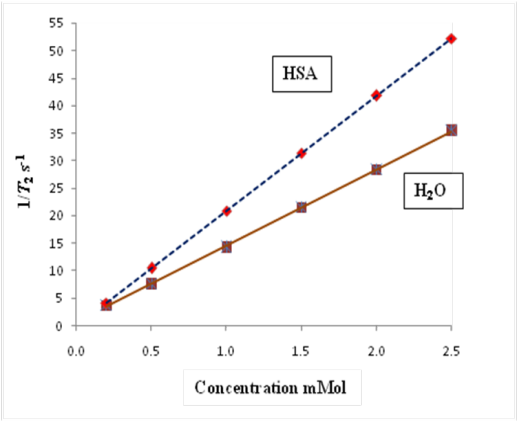


Figure 4. Transverse relaxivity of the complex (II) in water and HSA.

5.5 pH Dependent relaxivity of mono- and di-nuclear complex

The complex [Gd(DO3VA)$(H_2O)_2$] exhibits longitudinal relaxivities (r_{1p}) of 11.05, 11.31, 12.38, and 13.16 mM^{-1} s^{-1} at pH 2.6, 3.6, 4.6, and 5.6, respectively. These values are significantly higher than that of the complex in neat solution (r_{1p} = 5.32 mM^{-1} s^{-1}, pH = 7) (**Figure 5**). The relaxivity values at pH 7.2, 7.5, and 8.0 (TRIS-HCl buffer) are 4.90, 6.10, and 7.21, respectively. However, at pH 8.5, 9.6, and 10.6 the complex shows the longitudinal relaxivity of 0.90, 0.74, and 0.84, respectively. The di-nuclear complex also exhibits pH dependent longitudinal relaxivity of 21.38 and 21.16 mM^{-1} s^{-1} at pH 2.6 and 3.6, respectively, the relaxivity value gradually decreases on increasing the pH and reaches the minimum of 13.58 mM^{-1} s^{-1} at pH 7.2 and increases to 20.57 mM^{-1} s^{-1} at pH 10.6. The higher relaxivity at low pH may be caused by the protonation of the carboxylate oxygen, the nitrogen atoms of the ligand, and the water molecules hydrogen bonded to the periphery of the DO3VA chelates.

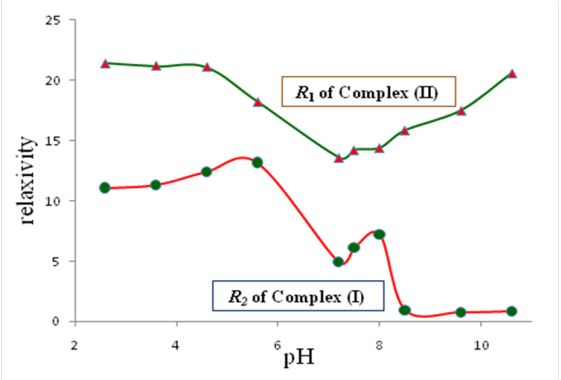


Figure 5. Longitudinal relaxivity of the complex (I) and (II) at various pH (2.6 to 10.6).

5.6 Relaxivity of [Gd(DO3VA)$(H_2O)_2$] and di-nuclear complex in the presence of HSA

The complex [Gd(DO3VA)$(H_2O)_2$] exhibits r_{1p} and r_{2p} values of 7.35 and 9.40 mM^{-1} s^{-1}, respectively, in the presence of HSA (1.36%) which is 1.38 and 1.22 times higher than that of the complex in neat aqueous solution (**Figure 1**). The di-nuclear complex displays r_{1p} and r_{2p} values of 18.55 and 20.89 mM^{-1} s^{-1}, respectively, in the presence of HSA which is 1.40 and 1.46 times higher than that for the neat solution (**Figure 2**). The higher relaxivity of the complexes is due to the binding of the 2-isovaleric acid pendant arm with the serum protein which results in a higher rotational correlation time for the macromolecular adduct. This promises their use as blood pool CAs for magnetic resonance angiography.

6. Conclusions

In the present investigation a convenient methodology has been developed for the synthesis of di-nuclear gadolinium(III) complexes of 1,4,7,10-tetraazacyclododecane-4,7,10-tris-2-iso valeric acid DO3VA] as contrast enhancing agents for MRI. The 2-isovaleric acid pendant arm appears to be a versatile moiety in the development of new contrast agents for MRI. A new classic draw near is established in developing a versatile polyfunctionable rigid amide linker and a di-nuclear complex with good “per Gd” relaxivity value. The coordination of amide functions on the linker shows a high thermodynamic stability and kinetic inertness of the agents, which are vital for avoiding transmetallation in vivo. No Gd^{3+} ions release was observed by UV–Vis spectrophotometry after a 4-week co-incubation of the agents with DTPA or endogenous ions such as Zn^{2+}, Ca^{2+} and Cu^{2+}, which reflected the very high kinetic inertia and thermodynamic stability of the macrocyclic gadolinium chelates. The pH-dependent relaxivity of the complexes could be exploited for their use as pH-responsive MRI contrast agents. Binding to HSA causes a considerable increase in the relaxivity of the monomer and the dimer as a result of slow rotational diffusion of the HSA-bound aduct. These complexes with the possible of binding with blood serum albumin through non-covalent interactions may present an extensive scope of evaluating their suitability as blood pool agents for

MRA. The synthetic strategies developed for the core molecule with amide linkers for anchoring gadolinium chelates can be exploited to develop higher generation dendrimeric CAs.

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