# Synthesis, pH and HSA Binding study of Novel Dinuclear [Gd(III)DO3VA] complex as Magnetic Resonance Imaging Contrast agent

**Abstract.** The synthesis and relaxivity study of a new gadolinium(III) complex of a cyclen macrocycle having 2-bromoisovaleric acid pendant arm (DO3VA) and its dinuclear complex is reported. The longitudinal relaxivity (r1p) of [Gd2{VA-acamido-pn(DO3VA)2}(H2O)2] is 19.90 mM-1 s-1 which correspond to a "per Gd" relaxivity of 9.95 mM-1 s-1. The "per Gd" r1p value is higher than that of the clinically approved CAs. The r2p value of the dinuclear complex is 21.35 mM-1 s-1 which correspond to a "per Gd" relaxivity of 10.67 mM-1 s-1. The "per Gd" r2p value of the complex is higher than that of the parent complex [Gd(DO3VA)(H2O)2]. The r2p/r1p value of 1.07 for the complex indicates that the complex is a T1-weighted CA. The complex exhibit pH-dependent r1p and r2p value at pH 2.6-10.6 indicating their potential use as pH-responsive contrast agent for MRI. The complex exhibit higher r1p value of 55.69 mM-1 s-1 in the presence of HSA indicating the formation of macromolecular adduct with HSA. The amide linker seems to be an excellent linker for making new CAs for MRA and dendrimeric CAs.

**Key words.** MRA Contrast agents, Amide linker, MRI, pH-responsive, DO3VA, HSA-Binding

## 1 INTRODUCTION

In the world of medicine, magnetic resonance imaging (MRI) is the most important and prominent technique in biomedical research.1 The 2003 Nobel Prize for medicine was awarded to Professor Paul C. Lauterbur (Biomedical Magnetic Resonance Laboratory, University of Illinois) and Professor Sir Peter Mansfield (University of Nottingham) for their seminal discoveries concerning the use of magnetic resonance to visualize different structures leading to the development of modern MRI.2 Due to high spatial resolution,3 MRI has become the clinical imaging procedure for a large number of studies involving the central nervous system, especially the brain,4 visualizing soft tissues,5 diagnosis of various diseases and the examination of almost all organs,6 and imaging of physiological properties such as diffusion, perfusion, and vascularity of tissues.3,7 MRI also provides real-time images of visualizing drug delivery,8 monitoring biological processes, and following functional changes in vivo.8,9 The image contrast obtained in MRI is a three-dimensional signal intensity map of the spatially encoded proton signal of the in vivo water molecules in a given volume element (voxel).10

Contrast in MRI images arises from variations in the relaxation times among water protons caused by differences in the local environment in tissues. For many clinical applications, it is a common practice to administer an exogenous chemical, called contrast enhancing agent, to improve the image contrast.10b Currently, about 30% of the MRI examinations use contrast agents.5,11

Contrast agents can be divided into two classes depending on whether they cause changes in longitudinal relaxation time (T1) or transverse relaxation time (T2) of the water protons, known as positive and negative agents, respectively.12 The relaxivity reflect the efficiency of a contrast agent to accelerate longitudinal and transverse water proton relaxation rates 1/T1 and 1/T2, respectively, normalized to one millimole concentration of the agent.10,13 The signal observed in MRI tends to increase with an increase in 1/T1 and decrease with an increase in 1/T2 and it is usual for contrast agents to affect both 1/T1 and 1/T2 to varying degrees.10 Positive contrast agents are commonly made up of paramagnetic materials, mainly those based on metal ions with large number of unpaired electrons such as Mn(II) and Gd(III). Positive contrast agents increase the brightness of images while the negative contrast agents give dark images. Superparamagnetic materials such as iron oxide nanoparticles are employed as negative CAs.1

Low molecular weight complexes of paramagnetic metal ions are administered as contrast agents prior to the acquisition of MRI images.1 Over the last decade there has been an increasing interest in the chemistry of Gd(III) complexes because they have been used in diagnostic medicine as contrast agents for MRI. The symmetric S-state of Gd(III) is a more hospitable environment for electron spins leading to a much slower electronic relaxation rate. Due to the large magnetic moment ($\mu eff2 = g2(S(S+1)) = 63$) with a long electron spin relaxation time (10-9 s) Gd(III) is widely used as a contrast agent for MRI.14 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.15 The commercially used contrast agents are Gd(III) complexes of polyaminocarboxylate ligands.

The choice of a contrast enhancing agent suitable

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for clinical use is governed by stringent biological requirements such as (i) high solubility in water (of the order of 0.5 M), (ii) effective catalyst for relaxation of protons in the bulk water, i.e., a high water proton relaxivity, (iii) better body distribution, (iv) low in vivo toxicity, i.e., extremely inert to the loss of the metal ion, (v) selective tissue/organ localization, (vi) rapid tissue clearance, and (vii) high thermodynamic stability and kinetic inertness. A sizable increase in relaxivity is achieved if Gd(III) chelates are covalently or noncovalently linked to macromolecules, because the rotational correlation time becomes comparable to or longer than the electronic and water-exchange correlation times.16 The conjugation of gadolinium(III)chelates to polymers, dendrimers, or biological molecules increases the rotational correlation time and improves the relaxivity "per Gd" atom. Targeting polynuclear conjugates are envisioned to provide MRI with the ability 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,17 proteins, and dendrimers.18

The mononuclear Gd(III) complexes of 2-iso valeric 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, the next largest class of applications may involve blood pool agents that serve as contrast agents for MRI angiography. Magnetic resonance angiography represents a more attractive procedure for patients and doctors for 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-d6 (99.9 atom % D), acetone-d6 (99.9 atom % D), D2O (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.15 HPLC grade water (Merck, India) was used to prepare solutions of the complexes for relaxivity measurements.

### 2.2 Preparation of buffer solutions

Glycine-HCl buffer for pH 2.6 and 3.6,16 glycine-NaOH buffer for pH 9.6 and 10.6,17 sodium acetate buffer for pH 4.6 and 5.6,17,18 TRIS-HCl buffer for pH 7.2, 7.5, 8.0, and 8.5 were prepared by the literature methods.

### 2.3 Preparation of 4.5% (w/v) human serum albumin (HSA) solution and complex/HSA samples

HSA was dissolved in PBS buffer (10 mM sodium phosphate and 150 mM sodium chloride, pH 7.4). An HSA molecular weight of 680,00 Da (Fluka) was used to convert % (w/v) to a molar concentration.

### 2.4 Binding of complexes to HAS

Gd complex/HSA samples ranging from 0.20 mM to 2.50 mM GdL and 0.066 mM (0.45%) to 0.66 mM (4.5%) HSA were prepared in PBS buffer. Each

complex/HSA sample was incubated at 37 oC for 1 h before T1 and T2 measurements.

## 3. METHODS

### 3.1 Physical Measurements

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR Spectrometer in the range of 4000-400 cm-1. Spectra for the solid samples were recorded by making transparent KBr pellets. The electrospray ionization mass spectra were recorded on a micromass Quattro II triple quadrupole mass spectrometer. The sample dissolved in methanol, acetonitrile, or water was introduced into the ESI source through a syringe pump at the rate of 5 µL per min. The ESI capillary was set at 3.5 kV and the cone voltage at 40 V. The average spectra of 6-8 scans were printed. 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. Caution! All hygroscopic compounds were dried in desiccator over silica gel for 24 h prior to the analysis. 1H and 13C 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 5mm 'QXI' was used for the 13C NMR measurements. 400 MHz 1H NMR spectra were recorded on a Jeol GSX-400 multinuclear NMR spectrometer working at 25 °C. The 100 MHz 13C NMR spectra were recorded using Jeol GSX-400 instrument. The standard 5 mm probe was used for the 1H and 13C NMR measurements. pH measurements were made using Labindia PICO+ pH/Conductive meter (3P) calibrated with buffer solutions of pH 4.0, 7.0, and 9.2.

### 3.2 Longitudinal relaxivity measurements

The longitudinal relaxivity of the Gd(III) complexes were determined from the spin lattice relaxation time (T1). The T1 measurements were carried out 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 T1 measurement. Solutions of six concentrations (0.2, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) for each complex were prepared in HPLC grade water (Merck, India) in 5 mL standard measuring flask (Vensil, Class "A"). The presence of free gadolinium(III) ion in the solution has been checked by xylenol orange test. The T1 measurements were made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection with τ values ranging from 50 µs to 6 s for each concentration of the complex.19 The computer program "Excel" (version 2007) was used to plot the time versus signal intensity to get a monoexponential plot and T1 was calculated from the plot. The T1 values for six concentrations of each complex were measured. The T1 curves for all the concentrations have a monoexponential decay character. The longitudinal relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex versus 1/T1 by least squares fitting method. The instrument was calibrated by measuring the relaxivity of [Gd(DO3A)(H2O)2] in aqueous solution (r1p = 4.63 mM-1 s-1, 20 MHz and 37 °C; lit.20 4.80 mM-1 s-1, 20 MHz, 40 °C).

### 3.3 Transverse relaxivity measurements

The transverse relaxation time (T2) was determined by the standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (90°-τ-180°) with a τ value of 50 µs.21 The computer program "Excel" (version 2007) was used to plot the time versus signal intensity to get an exponential curve and T2 was calculated from the graph. The T2 values for six concentrations were measured for each complex. The transverse relaxivity was calculated from the slope of the regression line, obtained by the plot of the concentration of the complex versus 1/T2 by least squares fitting method. All other experimental conditions are the same as that employed for the T1 measurements.

## 4 SYNTHESIS OF LIGANDS AND COMPLEX

### 4.1 4-Aza-1,7-bis(chloroacetamido)heptane (1)

It was synthesized by the procedure reported by Kubieck et al.24 White solid, yield (7.24 g) 85%, mp 120 °C. Anal. Calcd. for C10H19N3O2Cl2 (Mr = 284): C, 42.19; H, 6.54; N, 14.79. Found: C, 42.16; H, 6.53; N, 14.76. ESI MS: m/z 249 [M-Cl]+, 213 [M-2Cl]+, 173 [(M+2)-C2H2OCl2]+ (loss of two chlorine, one carbonyl, and one methylene group). 1H NMR (DMSO-d6, 278 K): δ 1.56 (4 H, m, CH2-aCH2-CH2), 2.88 (4 H, t, cCH2), 3.09 (4 H, t, dCH2), 3.51 (1 H, s. -NH), 4.05 (4 H, s, eCH2), 8.31 (2 H, s, HN-C=O). 13C NMR (DMSO-d6): δ 25.6 (CH2-aCH2-CH2), 29.1 (bCH2), 37.1 (cCH2-Cl), 43.1 (dCH2), 166.4 (HN-C=O).

### 4.2 4-Aza-1,7-bis[1-(1,4,7,10-tetraazacyclodo-decanyl)acet-amido]heptane (2)

A solution of 4-aza-1,7-bis(chloroacetamido) heptane (1) (0.72 g, 2.9 mmol) in 60 mL of water was added dropwise to a suspension of 1,4,7,10-tetraazacyclo-dodecane (cyclen) (1 g, 5.81 mmol) and sodium carbonate (anhydrous) (0.62 g, 5.81 mmol) in 100 mL of water. The reaction mixture was heated to 80 °C in an argon atmosphere for 24 h under stirring, cooled to room temperature, filtered, flash evaporated, washed with dry methanol, and dried in vacuum. The resulting white solid was recrystallized in water: yield (1.16 g,

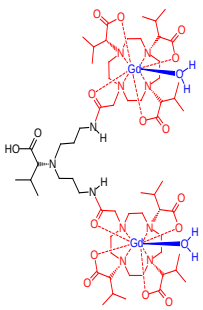
72%), mp > 210 °C (dec). Anal. calcd. for C26H57 N11O2 (Mr = 556): C, 56.19; H, 10.34; N, 27.72. Found: C, 56.21; H, 10.30; N, 27.67. IR (KBr, cm-1): 3417 ν(N-H), 2926 ν(C-H), 1657 ν(C=O), 1020 ν(C-N), 839 ω(N-H). MS (ESI): m/z 554 [M-2]+, 414 [M-C8H18N2]+.

### 4.3 {1,4,7,10-Tetraazacyclododecane-4,7,10-tris-2-iso-valeric acid} DO3VA (3)

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 DO3VA25 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 C23H44N4O6 (Mr = 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-CH2O3]+ (loss of one hydroxyl and one carboxyl group), 379 [(M-4)-C2H4O4]+ (loss of two carboxyl groups), 275 [(M+4)-C10H18O4]+ (loss of two isovaleric acid groups). 1H NMR (D2O, 278 K): δ 1.29 (9 H, d, aCH3), 1.40 (9 H, d, bCH3), 2.50 (3 H, m, CH3-cCH-CH3), 3.48 (16 H, t, dCH2), 4.41 (3 H, d, eCH). 13C NMR (D2O, 278 K): δ 15.8 (aCH3), 18.7 (bCH3), 28.1 (cCH), 31.5 (dCH2), 49.5 (eCH2), 52.6 (fCH2), 77.3 (gCH2), 181.1 (COOH).

### 4.4 4-Aza-(N-2-isovalericacid)-1,7-bis[1-(4,7,10-tris-2-isovalericacid-1,4,7,10-tetraazacyclododecanyl)acetamido]heptane [VA-acamido-pn (DO3VA)2, 4]

A solution of 2-bromoisovaleric acid (2.53 g, 14 mmol) in 40 mL of water was added dropwise at 55 °C over 1 h to a suspension of 4-aza-1,7-bis[1-(1,4,7,10-tetraazacyclododecanyl)acetamido] heptane (2) (1.04 g, 2 mmol) and sodium carbonate (1.48 g, 14 mmol) in 75 mL of water. The reaction mixture was refluxed under stirring in argon atmosphere for 40 h, cooled to room temperature, filtered, and flash evaporated to dryness.



[$Gd_2${VA-acamido-pn(DO3VA)$_2$}($H_2O$)$_2$] (5)

The resulting white hygroscopic solid was washed with dry methanol and recrystallized in water: yield (2.24 g, 89%), mp 280 °C (dec). Anal. calcd. for C61H113N11O16 (Mr = 1257): C, 58.30; H, 9.06;N, 12.20. Found: C, 58.33; H, 9.01; N, 12.19. IR (KBr, cm-1): 3448 ν(O-H) (acid), 2962 ν(C-H), 2853 ν(C-H), 1609 ν(C=O) (acid), 1256 δ(N-H), 738 ω(N-H), 586 ρ(CH3). MS (ESI): m/z 1239 [(M-1)-OH]+, 901 [(M-1)-C15H30O9]+.

### 4.5 [Gd2{VA-acamido-pn(DO3VA)2}(H2O)2] (5)

A solution of the ligand 4 (6.28 g, 5 mmol) in 40 mL of water was added to a solution of GdCl3.6H2O (3.72 g, 10 mmol) in 50 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 15 h. It was cooled to room temperature, filtered, and flash evaporated to dryness. The resulting colorless hygroscopic solid was purified by column chromatography by eluting with water and recrystallized in water: yield (6.56 g, 82%). Anal. calcd. for C61H111N11O18 Gd2 (Mr = 1601): C, 45.76; H, 6.93; N, 9.62. Found: C, 45.79; H, 6.91; N, 9.63. IR (KBr, cm-1): 3414 ν(O-H) (acid), 2971 ν(C-H), 1619 ν(C=O), 1298 δ(N-H), 1005 ν(C-N), 730 ρ(CH3), 427 ν(Gd-O). MS (ESI): m/z 1602 [M+1]+, 1590 [{(M+2)+Na}-2H2O]+, 1376 [(M-2)-GdC2H10O2]+.

## 5 Relaxivity Studies

### 5.1 Longitudinal relaxivity of [Gd2{VA-acamido-pn(DO3VA)2} (H2O)2]

The longitudinal relaxivity of the complex (5) in neat aqueous solution is 19.90 mM-1 s-1 (Figure-1) which corresponds to 9.95 "per Gd". The relaxivity is significantly higher than that of the parent complex [Gd(DO3VA)- (H2O)2] (r1p = 5.32 mM-1 s-1, 20 MHz, 37 °C) and the related dinuclear complex [Gd2{acamido-et(DO3VA)2}- (H2O)2] (r1p = 13.25 mM-1 s-1). The enhanced relaxivity is due to the combined effect of the presence of one inner-sphere water molecule and an increase in the molecular dimension. The higher r1p value of the system indicates the role of the linker in accelerating relaxivity. The presence of one 2-isovaleric acid in the linker and two replaceable hydrogen atoms contributes to the relaxivity enhancement.

### 5.2 Transverse relaxivity

The transverse relaxivity of [Gd2{VA-acetamido-pn(DO3VA)2}(H2O)2] is 21.35 mM-1 s-1 which corresponds to 10.67 "per Gd". The outer-sphere contribution to the overall relaxivity is attributed to the presence of a large number of water molecules hydrogen bonded on the periphery of the ligand framework. The importance of the spacer in accelerating relaxivity is also reflected in the r2p value.

The r2p/r1p ratio is 1.07 indicating that the complex is a T1-weighted contrast agent.

### 5.3 Relaxivity of the complex (5) in the presence of HSA

The complex [Gd2{VA-acetamido-pn(DO3VA)2}(H2O)2] exhibit r1p value of 55.69 and r2p value of 59.52 mM-1 s-1 in the presence of HSA (1.36%) which is about 2.80 times higher than that of the complex in neat aqueous solution (Figure-1). The higher relaxivity of the complex 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.

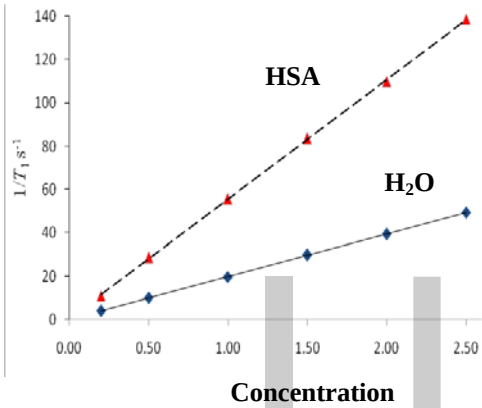



**Fig.1**. Longitudinal relaxivity of the complex (5) in water and HSA.

### 5.4 Relaxivity of the complex (5) at various pH

The dinuclear complex [Gd2{VA-acetamido-pn(DO3VA)2}(H2O)2] exhibits longitudinal relaxivities (r1p) of 25.8, 26.4, 28.97, 30.8, 11.47, 14.27, 16.87, 2.1, 1.73, and 1.96 mM-1 s-1 at pH 2.6, 3.6, 4.6, 5.6, 7.2, 7.5, 8, 8.5, 9.6, and 10.6, respectively (Figure-2). The relaxivity values are significantly changed when the solution pH is increased. The distinct change in the relaxivity values shows that the complex undergoes protonation at low pH. These pH responsive complexes could be good contrast agents in imaging the affected tissue in cancer diagnosis.

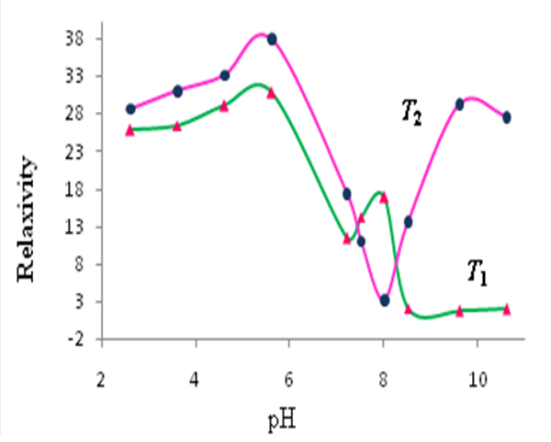



**Fig.2**. pH vs relaxitity (T1p and T2p) values of the complex (5)

## 6 Conclusions

In the present investigation a convenient methodology has been developed for the synthesis of dinuclear linker, ligand and their Gd(III) complex of DO3VA as contrast enhancing agents for MRA application. The higher relaxivity of the dinuclear complex indicate that each of the DO3VA moieties covalently appended to the linker or core molecule coordinates to the Gd(III) ion independently with inner-sphere water molecule(s). The outer-sphere contribution to the overall relaxivity is attributed to the presence of a large number of water molecules hydrogen bonded to the periphery of the hydrophilic 2-isovaleric acid groups.28 The amide linker molecules with ionizable protons appear to be quite suitable in the design of dinuclear Gd(III) chelate. It is interesting to note that the "per Gd" relaxivity increases with the nuclearity of the complex in addition to that an additional contribution to the overall relaxivity may arise from the paramagnetic interactions between the Gd(III) ions. Furthermore, the relaxivity depends on the nature of the linker/core molecules. The presence of the mobile protons on the linker molecule is found to have a profound effect in accelerating relaxivity. The 2-isovaleric acid pendant arm appears to be a versatile moiety in the development of new contrast agents for MRI. These complexes with the potential of binding with HSA through noncovalent interactions may offer a wide scope of evaluating their suitability as blood pool agents for magnetic resonance angiography (MRA). Also the complex shows the pH modulated relaxivity between 2.6 to 10.6 pH. The increase of pH upto 5.6 increases the relaxivity to 30.8 mM–1s–1. Hence it can be used as pH probes for determining the extracellular pH in tumors.

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