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LANTHANIDE(III) CHELATES AS MRI CONTRAST AGENTS: A BRIEF DESCRIPTION

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Magnetic resonance imaging (MRI) has become a prominent imaging technique in medicine. Gadolinium-based contrast agents are extensively used to enhance the contrast between normal and diseased tissues through MRI scans. The article illustrates the paramount significance of such contrast agents in MRI applications. Clinically approved contrast agents as well as those in trial period are discussed. Important parameters, i.e. hydration number, rotational correlation time, and mean residence lifetime, influencing the relaxivity (sensitivity) of such agents are described in detail. Various approaches towards relaxivity enhancement are discussed with appropriate examples from the recent literature. A decrease in the Gd-water proton distance results in significant relaxivity enhancement. A comprehensive classification and explanation of Gd^{3+}-based contrast agents are presented. Each class is explained with suitable examples. The stability of contrast agents is dependent on their chemical structure. Future contrast agents need to be tissue specific of high relaxivity, low toxicity, and lower administered dose for *in vivo* use.

K e y w o r d s: magnetic resonance imaging (MRI), gadolinium, relaxivity, nuclear relaxation, mean residence lifetime, contrast agents, hydration number, nephrogenic systemic fibrosis (NSF).

INTRODUCTION

Magnetic resonance imaging (MRI) is a noninvasive imaging modality used in modern clinical diagnosis to visualize a detailed internal structure and the limited function of the body. It is based on the detection of NMR signals emitted by protons of water and fat molecules in the body in a magnetic field. A magnetic field is applied to the region of interest, which is then modulated by radio waves. The nuclear energy states of protons interact with the incident radio waves. The radio frequency emission by tissue that follows the absorption of photons can be exploited to produce images [1—3]. In this method, three-dimensional images of the body parts are obtained in thin slices [4]. Paul C. Lauterbur and Sir Peter Mansfield won the Nobel Prize in 2003 for their work on MRI [5]. It is a useful medical technique for the diagnosis and monitoring of malignant tumors, cerebral abnormalities, multiple sclerosis, infarcted artery, and lesions. The key advantage of MRI is that it is safer for the patients than other imaging techniques such as X-rays where highly ionizing radiations are used for this purpose. Compared to computed tomography (CT), MRI provides much greater contrast between the different soft tissues of the body. In modern medicine, MRI has emerged as one of the most powerful techniques for the diagnosis and treatment of human diseases [6, 7].

The main challenge to this technique is, however, its low sensitivity. For clinical purposes hydrogen atoms of water molecules are considered, which are found in tissues at ~90 M. In order to improve the sensitivity of images obtained and enhance the contrast between normal and diseased tissues,

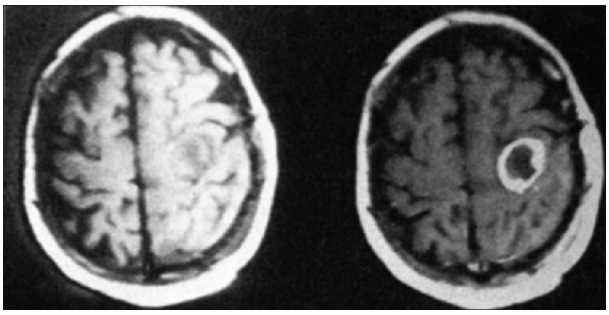


Fig. 1. Transverse NMR images (0.6 T, 24 MHZ) through the brain of a patient before (left) and 3 min later (right) the intravenous injection of $[Gd(DTPA)\times\times(H_2O)]^{2-}$ (dimeglumine salt; Schering/Berlex) at a dose of 0.1 mmol/kg. Characteristic ring enhancement of a tumor (high-grade astrocytoma) is seen in the postinjection image. Pulse sequence: spin echo TR = = 500, TE = 20 ms. (Courtesy of Dr. Thomas Brady, Massachusetts General Hospital) [1]

a substance is needed that will influence some property of 90 M water protons to an extent that there is an observable effect. Such substances are known as MRI contrast agents. These are paramagnetic, superparamagnetic, or ferromagnetic compounds that catalytically shorten the relaxation times of bulk water protons. The majority of these agents work through shortening the T_1 relaxation or spin-lattice relaxation time (a mechanism providing the thermodynamic equilibrium of the z component of the magnetization vector and its surroundings in MRI) of protons located in their surrounding. This T_1 shortening is due to an increase in the rate of stimulated emission from high energy states (proton spin aligned against the applied field) to low-energy states (proton spin aligned along the applied field). Contrast agents act as catalysts because they occur in a lower concentration as compared to the surrounding water. They act on the relaxation properties of water protons and thus create contrast between normal and diseased tissue. Nowadays, about 40—50 % MRI scans use contrast images. More than 10 million MRI scans are performed with gadolinium-based contrast agents each year [8—10].

TYPES OF MRI CONTRAST AGENTS

From the physical point of view, MRI contrast agents can be classified into two broad categories.

(1) T_1 agents which usually enhance the longitudinal (spin-lattice) relaxation rates ($1/T_1$) of water protons present in tissue more than the transverse (spin-spin) relaxation rates ($1/T_2$). The addition of such contrast agents causes the nuclei to appear as bright spots of increased intensity on T_1 weighted images due to the increased relaxation rate. Thus, these are positive contrast agents. Interaction between the excited nuclei and their surrounding environment gives rise to the T_1 (spin-lattice) relaxation process. Examples of such contrast agents are gadolinium or manganese chelates.

(2) T_2 agents, on the other hand, largely increase transverse (spin-spin) relaxation rates ($1/T_2$) and cause a decrease in signal intensity. Therefore, these are negative contrast agents. Relaxation caused by T_2 agents arises by the interaction between the excited nuclei and those with lower energy. Ferromagnetic iron oxide particles are T_2 agents [8, 11, 12].

The majority of MRI contrast agents are the complexes of the Gd^{3+} ion. The gadolinium ion lies at the midpoint of the lanthanide series. It has the maximum number of unpaired electrons (seven) among all the lanthanide ions and this, coupled with a long electronic relaxation time (7.9 BM), makes Gd^{3+} the best candidate as a relaxation agent. However, free Gd^{3+} is extremely toxic both *in vivo* as well as *in vitro* because its ionic radius (0.99 Å) is nearly equal to that of Ca^{2+}. Therefore, in living things, it competes with Ca^{2+}, resulting in its strong binding with biological systems where Ca^{2+} is needed. In order to ensure the safe clinical use of Gd^{3+}, it is complexed with organic ligands. It forms kinetically inert and thermodynamically stable complexes with some organic ligands. Multidentate ligands form stable complexes with Gd^{3+} because the chemical bonds in contrast agents are ionic in nature. Octadentate ligands are used for complexation with this trivalent ion. All of their eight donor atoms form coordinate bonds with Gd^{3+}. Since its coordination number is nine, therefore, the ninth coordinate bond is formed by a water molecule. Organic ligands together with a single water molecule attached directly to the central ion (Gd^{3+}) lie in the primary coordination sphere. Solvent molecules weakly interact with chelated Gd^{3+}, and thus, occupy the secondary coordination sphere. Diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetracarboxymethyl-1,4,7,10-tetraazacyclododecane (DOTA), and some of their derivatives have already been approved for clinical use because they form stable complexes with Gd^{3+} [13—15].

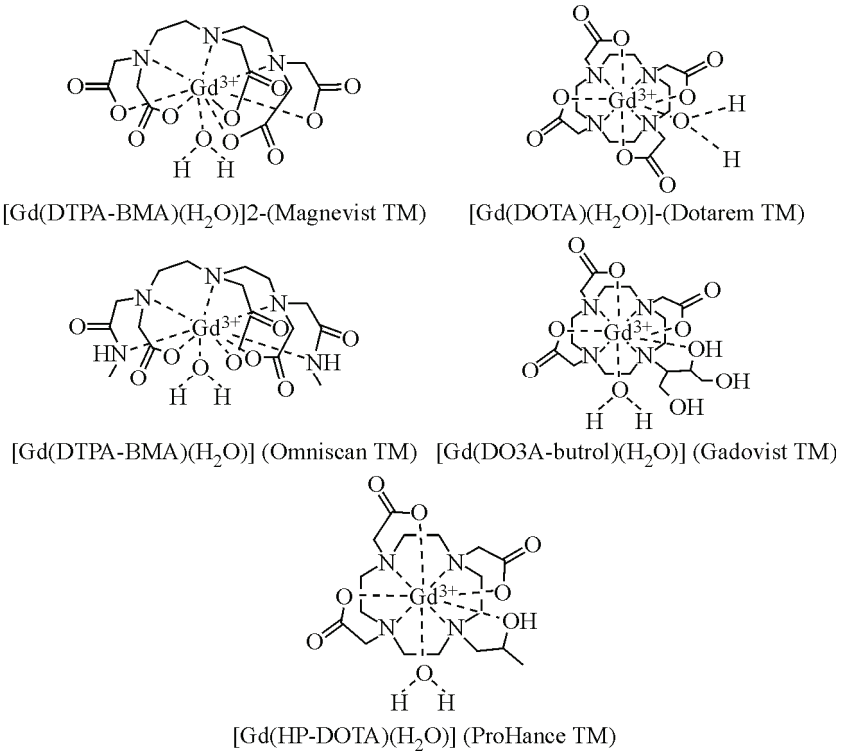


Fig. 2. Structure of some MRI contrast agents used for clinical purposes [13]

RELAXIVITY OF MRI CONTRAST AGENTS

The effectiveness of an MRI contrast agent is usually measured in terms of its relaxivity defined as the increase in water proton relaxation rate per unit concentration of a contrast agent. Contrast agents with high relaxivity cause greater contrast at equivalent doses than contrast agents with lower relaxivity [8, 13]. Solomon—Bloembergen theory describes the idea of nuclear relaxation. The observed relaxation rate of solvent protons (in the case of contrast agents/water protons) consists of both diamagnetic and paramagnetic contributions.

$$\frac{1}{T_{i,obs}} = \frac{1}{T_{i,d}} + \frac{1}{T_{i,p}}, \qquad i = 1, 2. \tag{1}$$

The paramagnetic contribution ($1/T_{i,p}$) is linearly related to the concentration of paramagnetic [Gd] species. Therefore,

$$\frac{1}{T_{i,obs}} = \frac{1}{T_{i,d}} + r_i[\mathrm{Gd}], \qquad i = 1, 2. \tag{2}$$

Relaxivity (r_i, i = 1, 2) is thus the slope of a plot of ($1/T_{i,obs}$) versus concentration. Therefore, in order to increase contrast in MR images, either a contrast agent of high relaxivity (r_i) should be used or an increased concentration of the local contrast agent would be needed [16, 17]. Dipole-dipole interactions between the proton nuclear spins and the fluctuating local magnetic field due to the unpaired electron spins of the paramagnetic substance (Gd^{3+}) cause the paramagnetic relaxation of water protons. Relaxation enhancement occurring due to the paramagnetic contribution consists of both inner-sphere component (caused by a water molecule coordinated directly with the Gd^{3+} ion) and outer-sphere component (caused by water molecules located in the secondary coordination sphere and the bulk solvent). Outer-sphere contributions are relatively small and are therefore neglected. Current research focuses on contrast agents of higher inner-sphere relaxivity from the protons of water molecules in the first coordination sphere of Gd^{3+}. The inner-sphere proton relaxivity arises from the chemical exchange of coordinated water protons with those in the bulk. The longitudinal ($1/T_1$) and transverse ($1/T_2$) relaxation rates of the bulk water nuclei are presented in Eqs. (3) and (4) respectively.

$$\left(\frac{1}{T_1}\right)^{IS} = qP_m\left[\frac{1}{(T_{1m}+\tau_m)}\right] \quad (3)$$

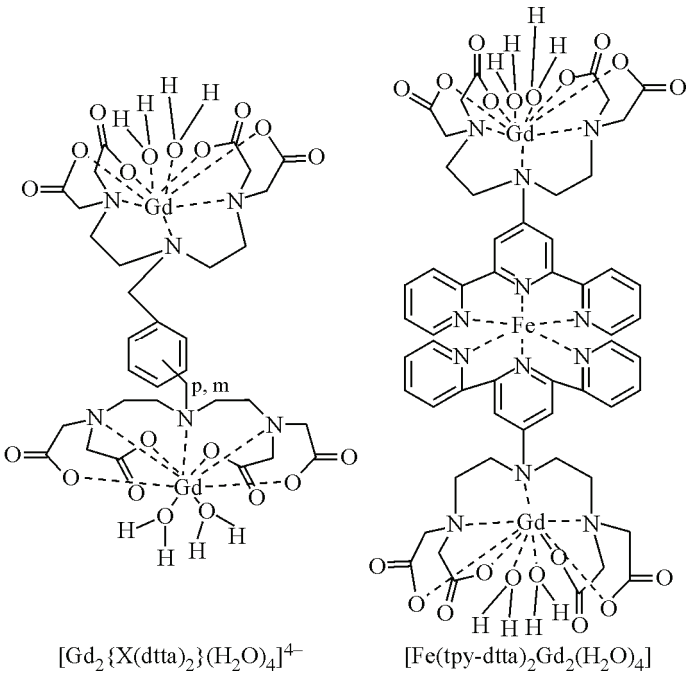
$$\left(\frac{1}{T_2}\right)^{IS} = \frac{P_m}{\tau_m}\frac{T_{2m}^{-2}+\tau_m^{-1}T_{2m}^{-1}+\Delta\omega_m^2}{(\tau_m^{-1}+T_{2m}^{-1})^2+\Delta\omega_m^2} \quad (4)$$

$1/T_{1m}$ and $1/T_{2m}$ are the longitudinal and transverse proton relaxation rates of the bound water respectively, q is the hydration number of Gd^{3+}, and P_m shows the mole fraction of coordinated water. τ_m is the mean residence lifetime, $\Delta\omega_m$ shows the chemical shift difference between the bound and bulk water molecules. It is evident from Eq. (3) that if the water exchange at the Gd^{3+} center is fast, the paramagnetic relaxation enhancement (experienced by the bulk solvent molecules) will arise from the longitudinal relaxation rate ($1/T_{1m}$) enhancement of the coordinated water molecules [17, 18].

According to Solomon and Bloembergen theory, three parameters largely affect the relaxivity of a contrast agent.

1. Hydration number (q). The inner-sphere proton relaxivity is linearly proportional to the hydration number (q) of Gd^{3+}. Therefore, a contrast agent of high relaxivity can be synthesized by increasing the hydration number of Gd^{+3}. For example, at 20 MHz for a complex with $q = 3$, the relaxivity can theoretically reach the values more than 300 $mM^{-1}s^{-1}$, showing a 60-fold increase over the relaxivities of current commercial contrast agents. However, the ligands accommodating more than one water molecule in the first coordination sphere when complexed with Gd^{3+}, exhibit reduced stability, and thus, their clinical safety also decreases. An increase in the hydration number also allows the coordination of other ligands, such as endogenous phosphate or bicarbonate, which then results in the displacement of coordinated water molecules and thus decreases the relaxivity. An example is the removal of an acetate ion from $[Gd(DOTA)(H_2O)]^-$, which then gives $[Gd(DO3A)(H_2O)_2]$ with $q = 2$, still a stable complex. $[Gd_2\{X(dtta)_2\}(H_2O)_4]^{2-}$ and $[Fe(tpy\text{-}dtta)_2Gd_2(H_2O)_4]$ are dinuclear Gd^{+3} complexes with $q = 2$. In both these examples, DTPA complexes are connected to a central core. These complexes show a significant increase in relaxivity compared to the parent DTPA complex ($r_{1p} = 4.3$ $mM^{-1}s^{-1}$) due to the increase in the hydration number of Gd^{3+}. $[Gd(DOTA)(H_2O)]^-$ and $[Gd_2\{X(dtta)_2\}(H_2O)_4]^{2-}$ possess the relaxivity values of 12.8 $mM^{-1}s^{-1}$ and 11.6 $mM^{-1}s^{-1}$ respectively (20 MHz, 37 °C). However, the thermodynamic stability of both these complexes greatly decreases. Therefore for safety reasons, complexes with $q = 1$ are preferred [18—20].

Among the contrast agents with $q = 1$, derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and systems based on the twisted square antiprismatic structure present a better choice. For such complexes, the exchange rate of coordinated water is faster and close to optimal values [21].



$[Gd_2\{X(dtta)_2\}(H_2O)_4]^{4-}$ $[Fe(tpy\text{-}dtta)_2Gd_2(H_2O)_4]$

Fig. 3. Examples of complexes with $q = 2$. Both complexes showed a significant increase in relaxivity [19]

2. Rotational correlation time (τ_R). It is another factor that affects the relaxivity of a complex. High relaxivity for a complex is achieved by lengthening the rotational correlation time (τ_R). It reflects the molecular tumbling rate. Longer values of τ_R improve the coupling of electron and nuclear spins at a magnetic field used typically in MRI (0.5—3 T). An increase in the hydrodynamic volume of the con-

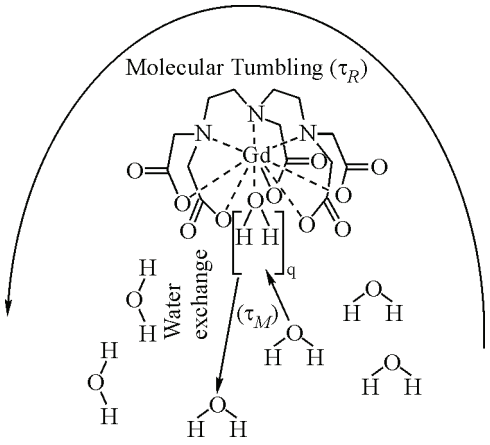


Fig. 4. Parameters that affect the proton relaxivity [19]

trast agent results in longer τ_R, which slows down the molecular tumbling [18]. This in turn generates a fluctuating magnetic field, inducing a relaxation of water molecules. For effective relaxation, the frequency of the fluctuating magnetic field ($1/\tau_R$ for small molecules) should be close to the Larmor frequency.

Small molecules have tumbling rates in gigahertz, whereas the Larmor frequency of scanners used in clinics ranges from 20 MHz to 130 MHz. High relaxivity can be achieved by slowing down the rotation, which is possible by increasing the contrast agent size through its coupling to a dendrimer or polymer or binding covalently or non-covalently to a protein [22]. However, the poorly controlled covalent addition of ligand molecules to proteins can result in a decreased thermodynamic stability of the complex. An alternative approach is the formation of the host-guest non-covalent interaction between the complex and a slowly tumbling macromolecule. Such an interaction causes a slow molecular tumbling and therefore lengthens τ_R. Complexes containing hydrophobic moieties can be coupled to a protein, such as human serum albumin (HSA). It is the most abundant protein in human blood plasma and contains a number of potential binding sites for hydrophobic moieties. Thus, it acts as a slow tumbling host for Gd^{3+} complexes. τ_R for small Gd^{3+} complexes has been reported in picoseconds. However, after binding them to HSA, τ_R is lengthened to nanoseconds, causing higher relaxivities. Gadofosveset trisodium [MS 325, MS 32520, Vasovist, ZK 236018] shown in Fig. 5 is a gadolinium-based blood pool contrast agent that can bind strongly and reversibly to HSA. The diphenylcyclohexyl group of this agent binds to the potential binding sites of HSA. As a result of strong HSA binding to Vasovist, the contrast agent spins much slower. HSA bound Vasovist thus shows a 60—100-fold increase in τ_R, causing its relaxivity enhancement at clinical field strengths. The relaxivity for this HSA bound agent has been reported to be 48.9 $mM^{-1}s^{-1}$ (20 MHz, 37 °C), which shows a 10-fold increase compared to low molecular weight chelates. A low dose (10—25 μmol/kg) therefore produces excellent blood tissue contrast enhancement. B22956/1 (gadoletic acid, shown in Fig. 5) is another gadolinium chelate that has the ability to bind reversibly to HSA. It is a DTPA derivative coupled to deoxycholic acid. B22956/1 bound to HSA showed a significant increase in relaxivity as this binding reduced the molecular tumbling and lengthened τ_R. HSA binding also resulted in a longer blood half-life of the contrast agent in the body.

Aime *et al.* reported a novel approach to lengthen τ_R by trapping a contrast agent $GdHPDO_3A$ in Apoferritin (ferritin without iron). Ferritin is a ubiquitous protein that stores iron and releases it in a

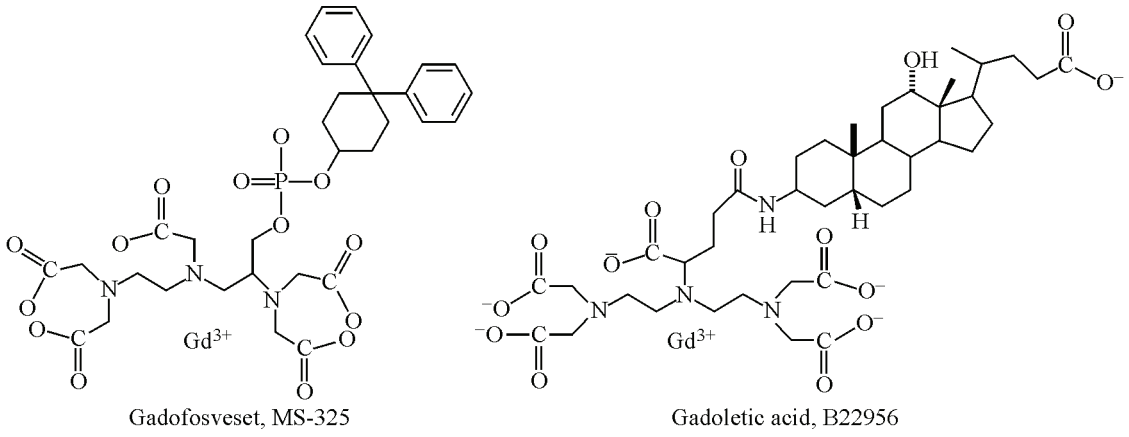


Fig. 5. Structures of Vasovist, MS-325, and B22956 [24]

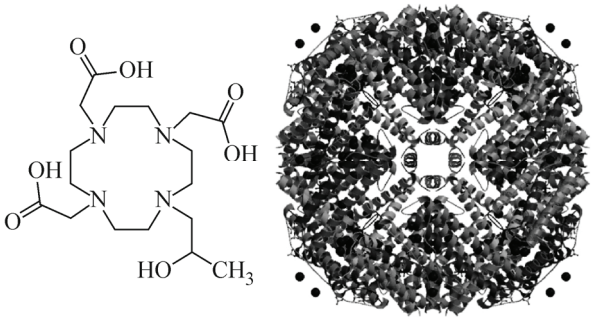


Fig. 6. Structures of $HPDO_3A$ [23] and the Ferritin complex [26]

controlled way. It consists of spherical compartments. A single ferritin molecule can accommodate about 4500 iron atoms in these spherical compartments. Apoferritin can accommodate several molecules of $GdHPDO_3A$. In their experiments, Aime and his coworkers allowed a solution of apoferritin subunits to assemble in the presence of excess of $GdHPDO_3A$. After exhaustive dialysis the solution was analyzed and apoferritin was found to contain 10 molecules of $GdHPDO_3A$ trapped in it. A large increase in the relaxivity (from 4.2 $mM^{-1}s^{-1}$ to 80 $mM^{-1}s^{-1}$ at 20 MHz and 298 K) of the complex was observed. A large spherical size of the complex caused its slow tumbling and longer τ_R, thus leading to high relaxivity [23—31].

Proton relaxation measurements are used in order the determine the τ_R values for Gd^{3+} complexes. However, this method does not give accurate values because there are a few factors that affect the proton relaxation measurements. For spherical molecules, the Debye—Stokes equation (Eq. 5) can be used to calculate τ_R.

$$\tau_R = \frac{4\pi a^3 \eta}{3KT}. \tag{5}$$

Here a is the radius of the spherical molecule, η shows the microviscosity of the medium, K indicates the Boltzmann constant, and T is the absolute temperature. This equation shows that τ_R is directly proportional to the viscosity of the medium η and the third power of the radius a. Although the correct values of η and a are difficult to determine, still this equation can be used to compare the rotational correlation times of complexes of different molecular weights, assuming that the microviscosity and the density of the molecules are the same; thus, the ratio of the cubed radii corresponds to the ratio of the molecular weights [32, 33].

3. Mean residence lifetime (τ_m). This parameter represents the lifetime of a water molecule coordinated to Gd^{3+} in the inner sphere of the complex. It is one of the most important parameters which influence the relaxivity of contrast agents. It also contributes to the overall τ_R of the complex. Longer τ_R values will have a maximum effect when the water residence lifetime (τ_m) of the coordinated water molecule is optimized. Water exchange at the Gd^{3+} center occurs by dissociation, i.e. a water molecule attached to Gd^{3+} leaves the inner coordination sphere, changing the complex to octadentate. However, another water molecule from the second coordination sphere replaces it. If the water exchange rate ($K_{ex} = 1/\tau_m$) is too slow, then the water molecule needlessly occupies the coordination site on Gd^{3+} and thus inhibits the relaxation of other water molecules in the second coordination sphere as well as in the bulk solvent. On the other hand, if the water exchange is too fast, then τ_m is short and therefore the water molecule does not spend enough time in the inner coordination sphere. Thus, water protons leave the inner coordination sphere without being relaxed effectively. Due to the dissociative mechanism of the water exchange, the energy difference between the ground state of the ennea-coordinated complex and its octa-coordinated transition state determines the water exchange rate. The introduction of bulky groups on DTPA, DOTA, and their derivatives induces strain in

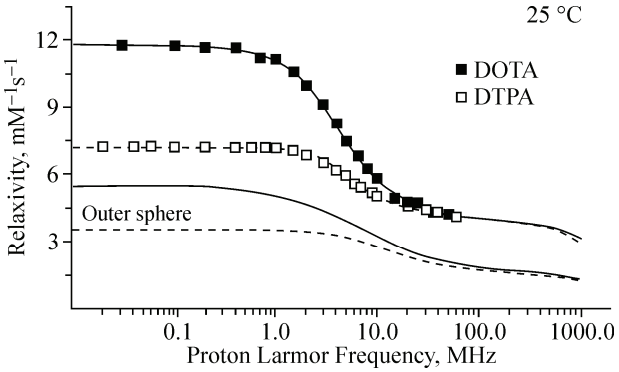


Fig. 7. $1/T_1$ nuclear magnetic resonance dispersion profiles of $Gd(DTPA)^{2-}$ and $Gd(DOTA)^{-}$ at 25 °C. The lower curves show the outer-sphere contribution [30]

the coordination cage. This strain causes the shortening of τ_m because the stability of the ground state decreases compared to the parent complexes. The water exchange rate (K_{ex}) for the Gd-based contrast agents presently in use is not optimal [18, 22, 29, 34, 35]. The τ_m value for the T_1 contrast agents currently used in clinics has been reported to be more than 100 ns. Its optimal value should be 10 ns at the most common magnetic field (1.5 T) now used in MRI scanners [36]. The electron density on Gd^{3+} affects the water exchange rate. Since Gd^{3+} is electron deficient, therefore it electrostatically attracts the lone pair of water oxygen. This causes the shortening of the Gd—O bond in the complex, resulting in the slow dissociation of the water molecule. However, if the donor atoms of anionic ligands donate electrons to the Gd^{3+} center, the demand to accept the electron pair from water oxygen decreases. Therefore, the complexation of ligands containing negatively charged donor atoms (CH_2COO^-) to the Gd^{3+} center would lead to a faster water exchange and a shorter mean residence lifetime of the water molecule in the inner coordination sphere. However, the water exchange rate is expected to be slower for neutral ligands (amides, alcohols). Sherry *et al.* reported that an increase in the number of ionic groups in a complex significantly shortened the mean residence lifetime of the water molecule. In their experiments, they replaced neutral amide ligating donors by anionic acetate donors. Their results are presented in Table 1 [29].

T a b l e 1

Increase in the number of acetates in the complex shortened the water residence lifetime [29]

S.NO	Complex	No. of acetates	τ_m^{298}, μs
1	GdDOTA-4AmP^{5-}	0	26
2	GdDOTA-2AmP^{3-}	2	6.2
3	GdDOTA-1AmP^{2-}	3	1.3
4	GdDOTA$^-$	4	0.24

Similarly, structure modifications that cause steric compression around the Gd^{3+} center also result in a more rapid water exchange. Merbach and his coworkers induced steric compression in poly(aminocarboxylate) ligands such as DOTA and DTPA. They replaced ethylene by a propylene bridge in order to achieve steric compression. The replacement of acetate by a propionate coordinating group also produced the desired results. Although this modification reduced the thermodynamic stability of the resultant complexes, however, they were stable enough to be used for clinical purposes [37]. The replacement of acetates by phosphonate groups in DOTA and its derivatives also increases steric hindrance. As a result, a water molecule can not approach closely to Gd^{+3} and thus, it is held at a greater distance. The water molecule, therefore, forms a weaker interaction with Gd^{+3}. This results in a faster exchange of coordinated water. However, the main drawback of phosphonate substitution is that the exchange rate becomes too fast. In this substitution, the O—Gd—O bond angle must be greater than 136°, otherwise phosphonate groups force the water molecule off the complex altogether [29]. For Gd^{3+} complexes, the water exchange rate is determined by measuring the transverse ^{17}O relaxation rate and the chemical shift of the water signal in their concentrated aqueous solution [33]. Swift and Connick described the relationship (Eq. 6) of paramagnetic transverse ^{17}O relaxation rate enhancement ($1/T_{2r}$) with τ_m and the relaxation rate of the coordinated water molecule ($1/T_{2m}$).

$$\frac{1}{T_2} - \frac{1}{T_{2A}} = \frac{P_m}{T_{2r}} = P_m \left[\frac{1}{\tau_m} \frac{(1/\tau_m + 1/T_{2m})/T_{2m} + (\Delta\omega_m)^2}{(1/\tau_m + 1/T_{2m})^2 + (\Delta\omega_m)^2} + \frac{1}{T_{2OS}} \right], \quad (6)$$

$$\frac{1}{T_{2r}} = \left[\frac{1}{\tau_m} \frac{(1/\tau_m + 1/T_{2m})/T_{2m} + (\Delta\omega_m)^2}{(1/\tau_m + 1/T_{2m})^2 + (\Delta\omega_m)^2} + \frac{1}{T_{2OS}} \right]. \quad (7)$$

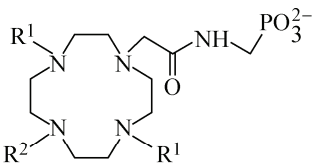


Fig. 8. Structure shows the replacement of neutral amide donors by anionic acetate donors

1. GdDOTA-4AmP^{5-}: $R^1 = R^2 = CH_2CONHCH_2PO_3^{2-}$
2. GdDOTA-2AmP^{3-}: $R^1 = CH2CO2- R^2 = CH_2CONHCH_2PO_3^{2-}$
3. GdDOTA-1AmP^{2-}, $R^1 = R^2 = CH_2CO^{2-}$

Table 2

Water exchange lifetime at 25 °C *for* Gd(III) *chelates as determined from the analysis of the temperature dependence of the water* ^{17}O *transverse relaxation rate* [30]

Ligand	Q	Charge	τ_m, ns	Ligand	Q	Charge	τ_m, ns
Aquo-ion	8	3+	1.2	DTPA-BMA [d]	1	0	2200
DTPA [a]	1	2–	303	DOTMA [e]	1	1–	68
DOTA [b]	1	1–	244	DTMA [f]	1	3+	19000
HP-DO3A [c]	1	0	350				

[a] Diethylenetriaminopentaacetic acid.
[b] 1,4,7,10-Tetraazacyclododecan-1,4,7,10-tetraacetic acid.
[c] 10-(2-Hydroxypropyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid.
[d] N,N-bis[2-[carboxymethyl[(methylcarbamoyl)methyl]amino]ethyl]glycine.
[e] (α,α″,α″,α‴)-Tetramethyl-1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid.
[f] 1,4,7,10-Tetrakis-[(N-methylcarbamoyl)methyl]-1,4,7,10-tetraazacyclododecane.

In the above equations, $1/T_2$ and $1/T_{2A}$ represent the ^{17}O NMR relaxation rates of the paramagnetic solution and of the external standard respectively. For the ^{17}O NMR relaxation rates, the outer-sphere contributions are negligible. Similarly, $\Delta\omega_m^2$ is also negligible compared to other terms in Eq. (7), which on simplification then gives Eq. (8).

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m}. \tag{8}$$

For paramagnetic transverse ^{17}O relaxation, the scalar contribution is the most important; therefore, it can be expressed as

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2sc}} = \frac{S(S+1)}{3}\left(\frac{A}{h}\right)^2\left(\tau_{E1} + \frac{\tau_{E2}}{1+\omega_s^2\tau_{E2}^2}\right). \tag{9}$$

The scalar coupling constant (A/h) in the above equation is determined from chemical shift measurements. In this equation, ω_s is the Larmor frequency, S is the electron spin quantum number (7/2 for Gd^{3+}), and τ_E represents the correlation time of the process modulating the scalar interaction. τ_m is described by the Eyring equation (Eq. 10)

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_b T}{h}\exp\left(\frac{\Delta S^\dagger}{R} - \frac{\Delta H^\dagger}{RT}\right), \tag{10}$$

where $\Delta S^\dagger$ and $\Delta H^\dagger$ are the activation entropy and the activation enthalpy respectively [17, 34, 35, 38, 39].

4. Gd—H distance (r_{GdH}). Since the inner-sphere relaxivity depends on $\frac{1}{r_{GdH}^6}$, therefore the Gd-water proton distance is important in the determination of the efficacy of a contrast agent. If r_{GdH} is decreased, it would cause a noticeable increase in relaxivity. However, it is a difficult parameter to determine experimentally. Generally, it is determined from the Gd-coordinated water oxygen distance. However, this procedure does not give accurate values of r_{GdH} because the tilt angle of the plane of the bound water molecule with respect to the Gd—O bond is ambiguous in solution. The reported in the literature values of r_{GdH} for Gd^{3+} chelates range from 2.5 Å to 3.3 Å. These values are also obtained indirectly from fitting nuclear magnetic relaxation dispersion (NMRD) data. Therefore, the r_{GdH} values calculated by this method are likely to be inaccurate because r_{GdH} also depends on the precise determination of the outer and second sphere contributions as well as it is correlated with the overall correlation time. The Gd—H distance is determined directly by the use of neutron diffraction on single crystals or isotopic exchange methods in a very concentrated solution of contrast agents. Electron nuclear

double resonance (ENDOR) spectroscopy is also used for the direct measurement of this parameter. Caravan and his coworkers measured the values of this parameter for the aqua ion and four commercial Gd^{3+}-based contrast agents from pulsed ENDOR spectra. They found the distance to be the same (3.1 ± 0.1 Å) for all the five complexes. Therefore, relaxivity enhancement appears to be difficult with the optimization of this parameter [8, 17, 40].

CLASSES OF CONTRAST AGENTS

In this section, a short description of the general classes of Gd-based contrast agents is presented. The majority of these contrast agents have been approved for human use and are currently used in clinics for recording MR images.

(1) Extracellular contrast agents. These contrast agents are low molecular weight gadolinium chelates that do not have tissue specificity. They are intravenously injected to patients prior to the MRI examination and are distributed nonspecifically throughout the plasma and interstitial spaces in the body. They show similar pharmacokinetics to iodinated radiographic contrast agents and are excreted by glomerular filtration via kidneys with an elimination half-life of about 90 minutes. Due to their small size, they rapidly diffuse from intravascular space into the interstitial space, therefore making biodistribution non-specific. Gd-DTPA (Magnevist) and Gd-DTPA-BMA (OmniScan) are the acyclic examples of this class of contrast agents. While macrocyclic chelates derived from 1,4,7,10-tetraazacyclododecane (cyclene) include Gd-HP-DO3A (Prohance) and Gd-DOTA (Dotarem). Gd-DTPA (Magnevist) was the first acyclic contrast agent to be approved in 1988 for clinical use. The stability of extracellular contrast agents is determined by their kinetic inertness and thermodynamic stability. Macrocyclic chelates are much more stable than linear, acyclic chelates. Among the macrocyclic complexes, Gd-DOTA (Dotarem) exhibits phenomenal kinetic inertness and high thermodynamic stability. For the majority of clinical examinations, the recommended dose of these contrast agents is about 0.1 mmol/kg of the body weight. However, for magnetic resonance angiography (MRA) and central nervous system (CNS) imaging, a higher dose (0.2—0.3 mmol/kg) might be required. Applications of

Table 3

Thermodynamic stabilities and relaxivities of commercial MRI contrast agents [41]

Chemical Name	Generic name	Brand Name	Company	1/*T* relaxivity, $mM^{-1}s^{-1}$	LogK, (therm)
$[Gd(DOTA)(H^2O)]^{2-}$	Gadopentate dimeglumine	Magnevist®	Schering (Germany)	4.30 [b,d]	22.46 [i]
$[Gd(DOTA)(H_2O)]^{-}$	Gadoterate meglumine	Dotarem®	Guerbet (France)	4.20 [b,d]	25.30 [j]
$[Gd(DTPA\text{-}BMA))(H_2O)]$	Gadodiamide	Omniscan®	Nycomed (Norway)	4.39 [b,e]	16.85 [k]
$[Gd(HP\text{-}DO3A)(H_2O)]$	Gadoteridol	ProHance®	Bracco (Italy)	3.70 [c,f]	23.80 [j]
$[Gd(DO3A\text{-}butrol)(H_2O)]$	Gadobutrol	Gadovist®	Schering (Germany)	3.60 [c,g]	21.80 [l]
$[Gd(DTPA\text{-}BMEA)(H_2O)]$	Gadoversetamide	OptiMARK®	Mallinckrodt (US)	4.70 [c,h]	16.84 [j]

[b] Relaxivities in water at 20 MHz and 25 °C.
[c] Relaxivities in water at 20 MHz and 40 °C.
[d] *Aime et al.* // Magn. Reson. Chem. – 1991. – **29**. – P. 923 – 7.
[e] *Powell et al.* // J. Amer. Chem. Soc. – 1996. – **118**. – P. 9333 – 9346.
[f] *Shukla et al*. // Magn. Reson. Chem. – 1996. – **35**. – P. 928 – 935.
[g] *Vogler et al.* // Eur. J. Radiol. – 1995. – **21**. – P. 1 – 10.
[h] *Periasamy et al.* // Invest Radiol. – 1991. – **26**. – P. S217 – S220.
[i] *Smith et al*. NIST Standard Reference Database# 46, 3.0 ed., 1997.
[j] *Kumar et al.* // Inorg. Chem. – 1994. – **33**. – P. 3567 – 3575.
[k] *Cacheris et al.* // Magn. Reson. Imaging. – 1990. – **8**. – P. 467 – 481.
[l] *Toth et al*. // Inorg. Chim. Acta. – 1996. – **249**. – P. 191 – 199.

these agents include the detection of tumors in the brain and faster imaging during the injection to get images of arteries or the blood flow to the heart [32, 41—43]. Clinically recommended doses of these contrast agents are usually safe and their side effects are rare. However, one of the serious adverse reactions caused by the use of Gd-based extracellular contrast agents is nephrogenic systemic fibrosis (NSF). It is a systemic fibrosing disorder characterized by widespread tissue fibrosis. This condition has been reported only in patients with kidney diseases. NSF chiefly affects the skin of the patient. However, it has recently been known that other organs of the body, such as liver, lungs, muscles, diaphragm, esophagus, and heart, may be involved. This disorder has been characterized by constant pain, muscle restlessness, and loss of skin flexibility. NSF is caused by the use of kinetically less inert contrast agents that liberate Gd^{3+}, resulting in its accumulation in the kidneys of the patient. According to a recent survey, 95 % of the patients suffering from NSF, were exposed to gadolinium chelate within 2—3 months before the disease showed its symptoms. Further the majority of these patients (about 90 %) had received gadodiamide in the past during an MRI examination. However, there have been no reported cases of NSF in patients with a normal kidney function [24, 44—46].

(2) Blood pool contrast agents. Blood pool contrast agents (intravascular agents) have high molecular weights (> 20 KDa) and are larger in size than conventional extracellular contrast agents. They are designed for longer vascular retention and enhanced relaxivity during an MRI examination. These features make them useful for MRA. For this purpose, Gd^{3+} chelates are covalently linked to polymers, proteins, and dendrimers or bound non-covalently to endogenous plasma proteins such as HSA. Thus, high molecular weights of these agents ensure their retention in the intravascular system for a prolonged time and prevent their leakage into the interstitium [21, 41, 47]. These contrast agents were designed as the non-invasive replacement for X-ray angiography, a potentially dangerous technique to obtain high resolution images of arterial blockages in the body [32]. The following are the important types of these agents.

(a) Protein-binding gadolinium complexes. These contrast agents include low molecular weight complexes that bind non-covalently to plasma proteins. Such agents when coupled to protein show a longer intravascular retention. Unlike conventional extracellular agents, they exhibit fast renal excretion. A successful example of this group of agents is MS-325 Gd^{3+}. It binds strongly and reversibly to HSA in plasma, causing a significant increase in its relaxivity. It can bind at about 20 ± 5 sites of a single HSA molecule. This agent gives enough intravascular contrast for up to one hour. Other examples of such agents include B22956/1 (gadoletic acid) $[Gd(BOPTA)(H_2O)]^{2-}$ (Multihance^{TM}, Bracco), $[Gd(EOB\text{-}DTPA)—(H_2O)]^{2-}$ (Eovist^{TM} Schering), and MP-2269 (Mallinckrodt). Gd(EOB-DTPA) forms modest non-covalent association with a HSA molecule. It is being studied as a liver contrast agent due to its hepatocytes targeting ability. These agents have different protein binding abilities due to different substituents on the core chelate. For example, MS-325 has known to be 96.2 % protein bound in human plasma at a concentration of 0.1 mmol/l and 94.3 % bound in a 4.5 % HSA solution. Gd(EOB-DTPA), on the other hand, remains only 10 % protein bound in human plasma at a concentration of 1 mmol/l and 11 % bound in a 5 % HSA solution [25, 47—49]. B229561/1 has been tested for *in vitro* studies and it remains bound to a 4.5 % HSA solution at a drug concentration of 0.1 mmol [41] The binding affinity (K_A) of these agents is expressed as

$$K_A = \frac{[\mathrm{GdL} - \mathrm{HSA}]}{[\mathrm{GdL}][n\mathrm{HSA}]}. \tag{11}$$

The thermodynamic association constant of these complexes with HSA ranges from $10^2\ M^{-1}$ to $10^4\ M^{-1}$ [30, 31]. Apart from being used for MRA, these agents have useful applications in breast MRI, perfusion MRI, and lung perfusion. Such agents are also used for the imaging of malignant tumors since they accumulate in leaky vessels near tumor capillaries [24].

(b) Polymeric gadolinium complexes. Due to a large size, polymeric Gd^{3+} complexes leak slowly into the interstitial space through the endothelium of the vascular system, thus providing a prolonged imaging window. Some polymeric agents such as dextrans and polylysine derivatives have been examined. Their molecular weights vary from 15,000 to several million Da. A larger molecular size of the polymeric agents results in their high rotational correlation time, which in turn causes an

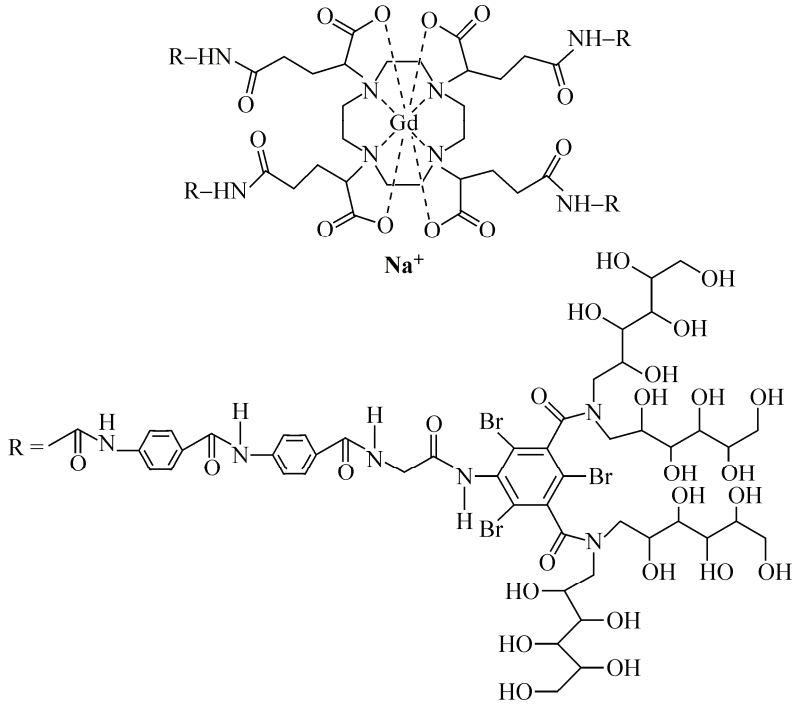


Fig. 9. Structure of P-792, Vistarem® [24]

increase in their relaxivity**.** Since their molecular weight is much greater than 40 kDa, they can stay in the vascular system for a longer time. However, their larger molecular weights lead to their slow renal excretion via glomerular filtration, which can cause safety problems. Gadomer 17 is a successful example of the polymeric gadolinium contrast agents. This agent has a medium size (molecular weight 35 kDa) and consists of 24 Gd^{3+} ions. It is large enough to exhibit a slower leakage through the normally functioning endothelium and small enough to be eliminated via glomerular filtration in the kidneys [24, 25]. It has been used for imaging animals (rat, dog, rabbit, monkey) producing good results. It was administered via intravenous injection (25—100 μmol/kg body weight). The studies showed that this agent was fully distributed within the intravascular space and did not exhibit a significant diffusion into the interstitial space. Further it did not accumulate in the body for a long time and was eliminated rapidly [50]. P-792 is another rapid clearance blood pool agent that has been examined even for human use. It is a high relaxivity blood pool contrast agent at clinical field strengths. In order to study its pharmacokinetics and safety, it was administered through intravenous injection (0.0065 mmol/kg, 0.013 mmol/kg, 0.026 mmol/kg, and 0.039 mmol/kg) to 32 healthy male volunteers. Some mild to moderate side effects were reported, but they resolved soon without any treatment. About 55 % of the injected dose was excreted in a couple of hours after intravenous injection, while 65—80 % of the dose were eliminated within 72 h. Only 0.10 % of P-792 was found in the urine of some of the volunteers after 22 days of the injection. The terminal half-life of this agent was reported to be 2 h. The pharmacokinetic properties of P-792 suggest that it could be a useful agent for MR coronary angiography, perfusion imaging (stress and rest), and permeability imaging (detection of ischemia and tumor grading). However, the research work is still being done in order to ensure its safe use in clinics [51, 52].

$(Gd\text{-}DTPA)_n$-polylysine is another polymeric contrast agent. It is still under development (PLLGd-DTPA, preclin, Bayer Schering Pharma AG). A successful synthesis of this agent was reported in 1988 for the first time. It consists of two step reactions, i.e. the binding of DTPA to polylysine producing a macromolecular ligand and then chelation of this ligand with gadolinium chloride. Spin-lattice and spin-spin relaxivities for $(Gd\text{-}DTPA)_n$-polylysine were found to be about three times larger than those of Gd-DTPA. This agent is expected to have useful applications in the perfu-

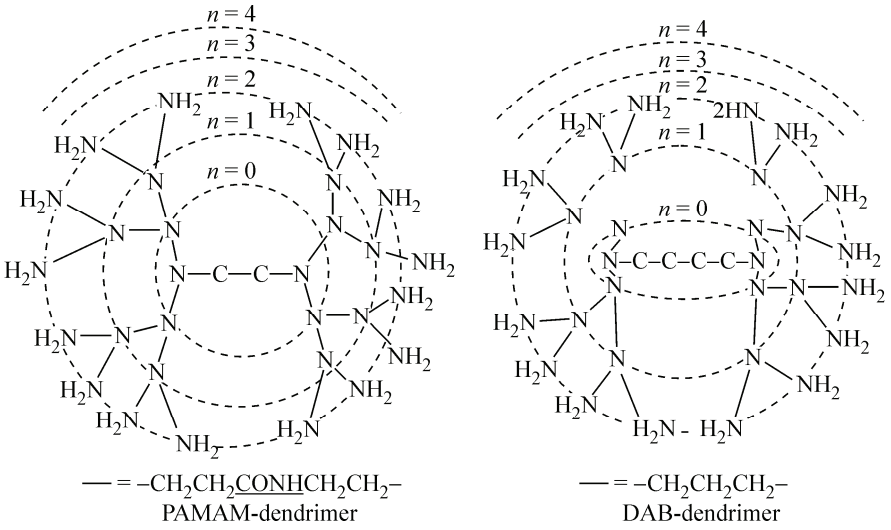


Fig. 10. Structures of commercially available dendrimers used as cores of contrast agents [54]

sion studies of the myocardium and lung perfusion, MRA, and differential diagnosis of tumors [24, 53].

(c) Dendrimer-based Gd^{3+} agents. Dendrimers or cascade polymers represent a class of repeatedly branched and roughly spherical large polymers. Polyamidoamine (PAMAM) and polypropylenimine diaminobutane (DAB) are the two commercially available dendrimers (Fig. 10). These dendrimers show high solubility in the aqueous solution and a single molecule of both these dendrimers has hundreds of possible sites for coupling to active species. A large number of amino groups are available on their surface, which makes them suitable substrates for coupling to chelating agents. Thus, gadolinium chelates are covalently attached to these sites on the surface, resulting in the formation of macromolecular MR contrast agents. Generally, DTPA and DOTA derivatives are used for coupling with dendrimer cores. Such agents can be synthesized in different sizes, depending on the number of successive branching reactions (generations). Kobayashi *et al.* reported the synthesis of novel dendrimer-based macromolecular MRI contrast agents with molecular sizes ranging from 3 nm to 15 nm and molecular weights ranging from 29 kDa to 3850 kDa. The chelating agents after being coupled to dendrimers, showed significant changes in blood retention, tissue perfusion, excretion rates and pathways. Further, they are likely to feature low polydispersity, no antigenicity, and relaxivity enhancement. Smaller-sized (3—6 nm in diameter) contrast agents having molecular weights less than 60 kDa were found to be suitable as functional renal contrast agents because they were rapidly excreted via kidneys. Larger-sized (7—12 nm in diameter) hydrophilic contrast agents are reported to be retained in the circulation and therefore suite well for the use as blood pool contrast agents. Hydrophobic variants formed with polypropylenimine diaminobutane (DAB) dendrimer cores were found to be liver contrast agents [54, 55].

(d) Ultrasmall superparamagnetic iron oxides (USPIOs). These are nanoparticles made of stabilized iron oxide. Such agents consist of a magnetite (Fe_3O_4) core (of approximately 5 nm in size) bound by a protective thin dextran coating. The overall size of USPIOs has been determined to be 21 nm. The research on their magnetic behavior showed that these agents exhibit the magnetic susceptibility in between paramagnetic and ferromagnetic substances. Due to a small size of the magnetite core, a magnetic moment is produced, which then experiences much less magnetic lattice anisotropy than that calculated for larger crystalline systems. This small magnetic anisotropy allows thermal reorientation of the magnetization vector, leading to the superparamagnetic behavior.

The net magnetization for such superparamagnetic agents is proportional to the magnetic field at low fields (Eq. 12).

$$M_s = F_s N \mu_s L(x), \qquad L(x) = \coth(x) - 1/x, \qquad x = \frac{\mu_s B}{KT}, \tag{12}$$

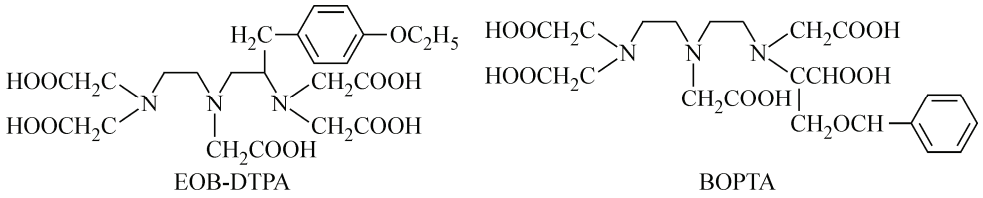


Fig. 11. Structure of EOB-DTPA and BOTPA [11]

N is the total number of iron atoms; F_S shows the number of magnetite cores divided by *N*; μ_S indicates the magnetic moment of the magnetite core; *K* represents the Boltzmann; *s* constant; *B* is the external magnetic field strength, and *T* shows the temperature. *L*(*x*) is known as the Langevin function. For $x << 1$, $L(x) = x/3$. In such a condition, M_s approaches zero as *B* approaches zero [47]. Examples of USPIO contrast agents include ferumoxtran-10 (Sinerem®, Guerbet = Combidex®, AMI 7227, Advanced Magnetics), NC 100150 (Amersham Health), SH U 555C (Schering AG), and VSOP-C184 (Ferropharm GmbH). Although these agents have been found to exhibit relatively lower relaxivities, however, their favorable T_1/T_2 ratio makes them suitable agents for MRA and other T_1-weighted MR techniques. A dose of about 25—50 μmol Fe/kg is needed in order to get adequate MRAs. These agents are excreted from circulation by their uptake into liver, spleen and lymph nodes through the reticuloendothelial system (RES). This mode of excretion takes a longer time than simple excretion via kidneys. Due to a small size, these agents accumulate slowly in RES, which leads to their long plasma half-life [24, 25].

(3) Targeted contrast agents. Blood pool agents showed similar behavior to the first-generation, low molecular weight contrast agents already in clinical use. They are preferentially distributed in the body due to their size. Therefore another approach is to enhance the targeting efficiency of contrast agents to obtain high signal intensity in the target tissues [13]. The term ″targeting″ is used for the selective and highly specific binding of a ligand (binder) to a molecule of interest (molecular target) *in vitro* or *in vivo*. For this purpose, contrast agents that are specific to organs, diseased tissues or that have the ability to recognize particular biochemicals are being developed. Such agents would enhance the efficiency of MRI due to the specific localization of molecular targets together with noninvasiveness and high spatial resolution of this imaging technique. However, high local concentrations of such contrast agents are required in the target molecules to produce detectable contrast. To overcome this problem, an efficient targeted contrast agent needs to have high affinity and specificity for targeted cells. These agents are administered to patients at a lower dosage than extracellular contrast agents. The relaxivity of these agents mainly depends on the number of contrast-generating groups per single molecule of the agent. Numerous targeted contrast agents have been synthesized by the introduction of suitable residues into either the acetic side-arms or diethylenetriamine backbone of Gd-DTPA and Gd-DOTA. Among the targeted agents, $[Gd(BOPTA)]^{2-}$ and [Gd(EOB-DTPA)] are DTPA derivatives with lipophilic side chains which have been reported to be liver specific. Macrocyclic Gd^{3+} complexes with a perfluorinated side chain have shown suitable characteristics for the use as lymph node specific agents. Both liver specific and lymph node specific agents accumulate in their respective target cells by the process of phagocytosis or endocytosis. Porphyrin-based Gd^{3+} complexes have been reported to accumulate in necrotic tissues and are therefore tumor specific. Similarly, contrast agents based on the conjugates of antibodies coupled to the conjugates of polymeric carriers have also exhibited the properties of tumor specific agents.

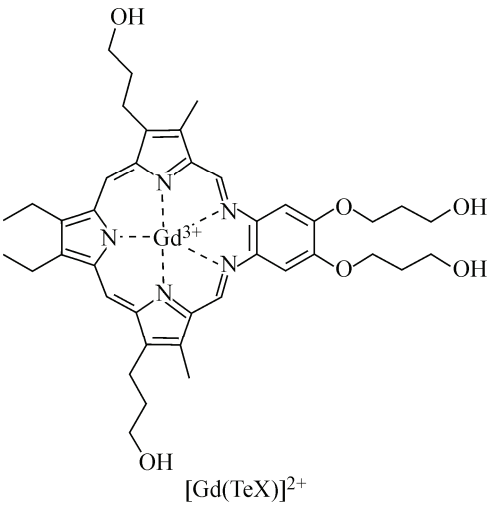


Fig. 12. $[Gd(TeX)]^{2+}$ structure [56]

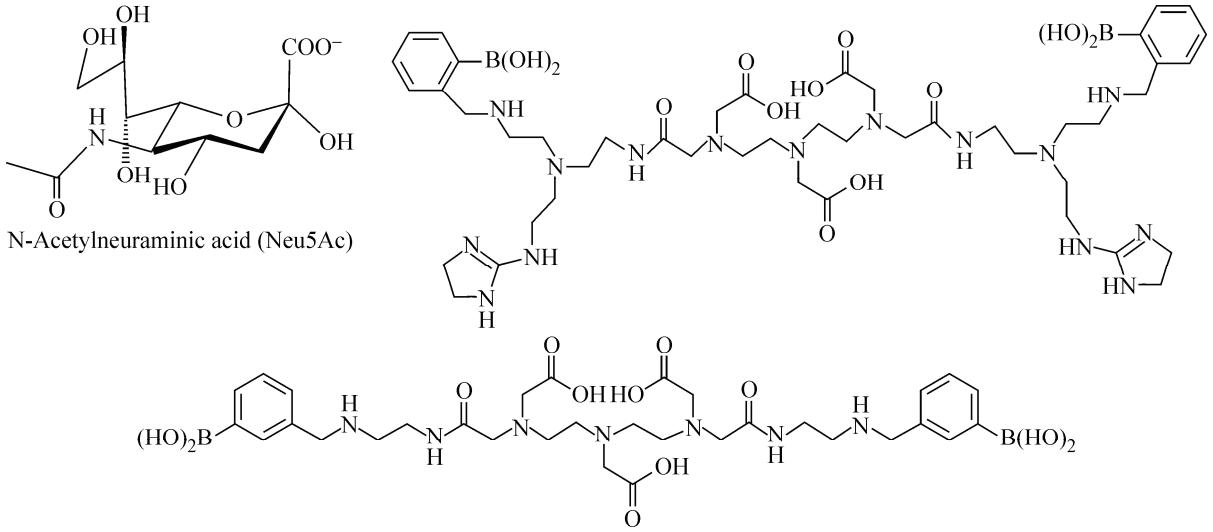


Fig. 13. Structures of N-acetylneuraminic acid (Neu5Ac) and two targeting ligands used for the malignant tumor cell surface [58]

Some of the tumor cells are known to contain high negative charges on their cell surface. Therefore, another approach to targeting tumor cells consists of a previous interaction of the tumor cell surface with a polypeptide formed by positively charged amino acids (polyarginine or polyornithine). These amino acids (due to their positively charged nature) are suitable to interact with a negatively charged DO3A derivative, containing an additional pendant arm with phosphonate and carboxylate groups added later.

Texaphyrin is a sub-class of heterocyclic macrocycle molecules called porphyrins. It consists of five nitrogen donor atoms. The Gd^{3+}complexes of texaphyrin have been reported to accumulate in tumors and atherosclerotic plaque. These agents are being examined in photosensitized and radiation sensitized treatment of cancer and cardiovascular diseases. These complexes are highly stable since the cavity has completely enclosed the Gd^{3+} ion. A long correlation time of $[Gd(TeX)]^{2+}$ results in its higher relaxivity than that of $Gd(DTPA)^{2-}$ and $Gd(DOTA)^{-}$ (3—4 times greater) [11, 56, 57].

Frullano and his coworkers synthesized two new ligands which can detect sialic acid residues on the surfaces of malignant tumor cells. Sialic acid is a generic term for the N- or O-substituted derivatives of neuraminic acid: a monosaccharide with a nine-carbon backbone. Malignant tumor cell surfaces contain a higher amount of sialic acid residues (> 10^9 Cell) than normal cells (~10^6 Cell). The ligands consist of DTPA-bisamide whose amide moieties consist of both a boronic functional group for the interaction with diol groups in the side chain of sialic acid and a positively charged functional group (physiological PH values) that interacts with the carboxylate anion of sialic acid [58].

Boutry *et al.* reported the synthesis of a new E-selectin-targeted contrast agent for the imaging of blood vessels in inflamed tissues. E-selectin or leukocyte-endothelial cell adhesion molecule 2 (LECAM2) is a cell adhesion molecule expressed only on the endothelial cells activated by cytokines. Like other selectins, it plays a key role in inflammation. It is found on sinusoidal lining cells in the inflammatory liver disease. The contrast agent Gd-DTPA-B(sLe^X)A mimics the Sialyl-$Lewis^X$ molecule, which is an E-selectin ligand. The contrast agent specifically targets the site of inflammation and accumulates there by interacting with E-selectin expressed on blood vessels (Fig. 14) [59].

An approach used for the accumulation of the contrast agent at the target site is the cell internalization process. In order to make this process effective, the agent concentration inside the cell needs to be higher than that at the cell surface. Such internalization processes can take place via receptor-mediated endocytosis or via pinocytosis and phagocytosis mechanisms. A suitable example of this process is the entrapment of Gd-$HPDO_3A$ units inside the inner spherical cavity of apoferritin. After intravenous administration of the contrast agent, specific receptors on hepatocytes rapidly clear it up [49].

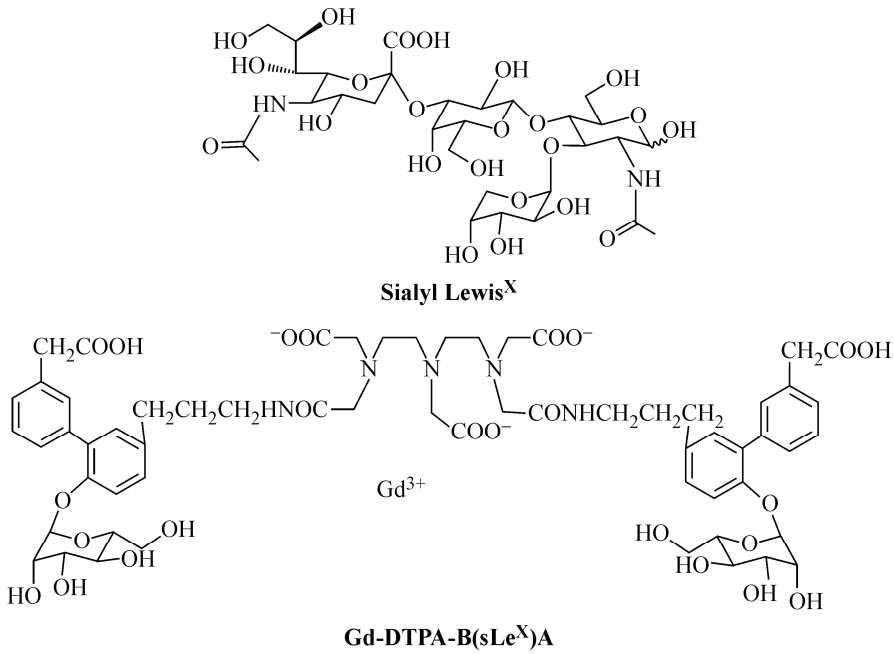


Fig. 14. Molecular structures of Sialyl-Lewis^X and E-selectin-targeted contrast agent Gd-DTPA-B(sLe^X)A [59]

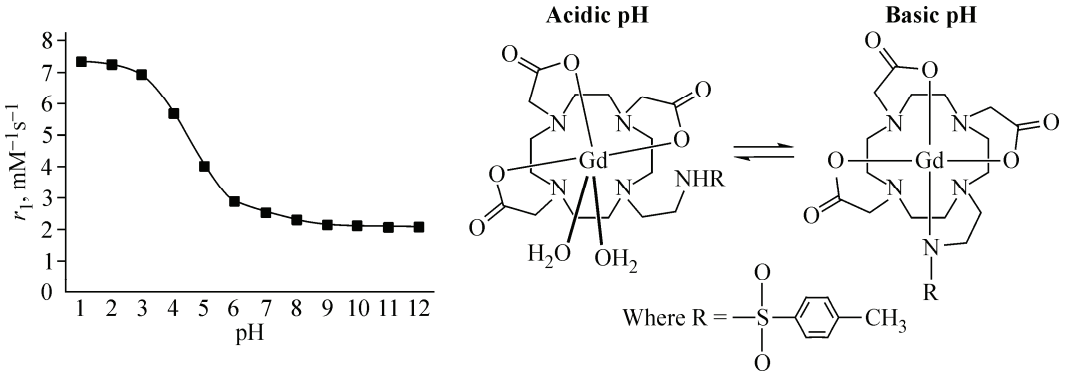


Fig. 15. pH dependence of the relaxivity of a Gd^{3+} complex bearing an arylsulfonamide group. (20 MHz, 25 °C) [33]

(4) Responsive contrast agents. Responsive contrast agents also referred as smart contrast agents undergo a large change in relaxivity as they respond to changes in their physiological environment. Typical parameters to which responsive contrast agents are sensitive include PH, metal ion concentration, oxygen pressure, and enzymatic activity.

pH Responsive contrast agents. These agents are of special interest since their relaxivity is pH-dependent around physiological pH values. Tumor tissues (PH = 6.8—6.9) are slightly more acidic than normal tissues (pH = 7.4). Therefore, pH responsive contrast agents can serve to distinguish tumor tissues from healthy tissues. The pH-dependent relaxivity of a complex arises either from changes in the hydration number of the complex or the protonation of a donor atom (such as O or N) in a ligand group. In the majority of reported Gd^{3+} complexes, the pH-dependence of the relaxivity hasbeen found to be related to changes in their hydration number. For example, the relaxivity of a Gd^{3+} complex bearing an arylsulfonamide

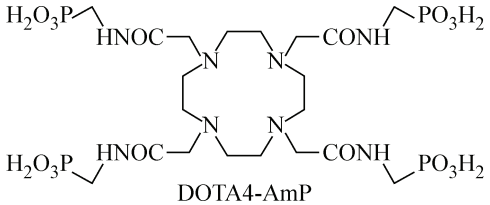


Fig. 16. DOTA4-AmP structure [49]

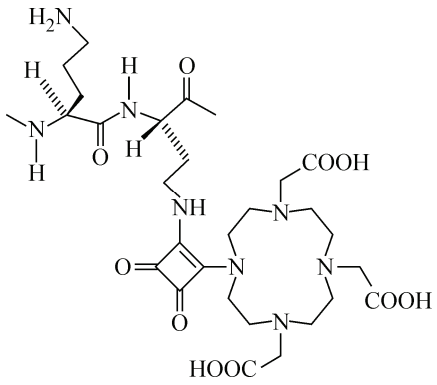


Fig. 17. Macromolecular construct formed by 30 Gd^{3+} units covalently linked by a squaric acid moiety to poly-ornithine [33]

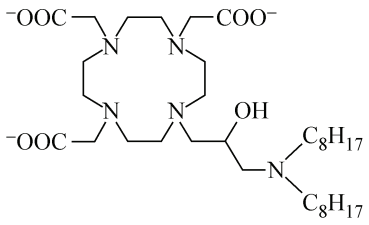


Fig. 18. Molecular structure of Gd-HADO-DO3A [60]

group, is reported to be significantly dependent on pH. The relaxivity of the complex at acidic pH (pH < 4) is 8 $mM^{-1}s^{-1}$, while it is reduced to about 2.2 $mM^{-1}s^{-1}$ at basic pH (pH > 8). The observed decrease in the relaxivity of the complex (about 4-fold) is due to a change in the number of water molecules coordinated to the Gd^{3+} ion from 2 (at acidic pH values) to zero (at basic pH values).

In some complexes, the pH-dependent relaxivity is related to changes in their second coordination sphere. Zhang *et al.* observed that the relaxivity of GdDOTA-4AmP (amino phosphonate tetraamide derivative) showed variation with pH. This pH dependence of the relaxivity for this complex is reported to be due to the formation/disruption of hydrogen bonds between the pendant phosphonate groups and water molecules bound to the Gd^{3+} ion.

Changes in the dynamic properties also influence the relaxivity of the complex. A suitable example is given by a macromolecular construct formed by 30 Gd^{3+} units covalently linked by a squaric acid moiety to poly-ornithine (Fig. 17). At lower pH, free amino groups are protonated and therefore, they tend to stay as far apart as possible, resulting in the low relaxivity of the complex. At high pH, the polymer assumes a rigid structure of high relaxivity owing to the formation of intramolecular hydrogen bonds between the adjacent peptidic linkages [23, 33, 49].

Hovland *et al.* synthesized Gd-HADO-DO3A, a pH responsive contrast agent which showed an increase of about 142 % in its relaxivity with changing pH from 6 to 8. The increase in the relaxivity by changing pH was suggested to arise from the formation of supramolecular structures caused by the deprotonation of the amphiphilic complex at basic pH [60].

Ali and his coworkers improved the pH-responsive action of a low molecular contrast agent (for example, Gd 11 in Fig. 19) by coupling it to the G5-PAMAM dendrimer. This conjugation caused a

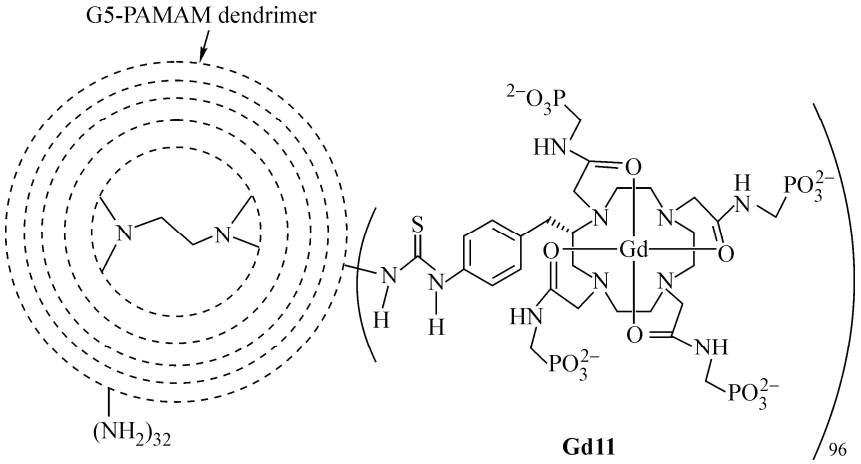


Fig. 19. Structure of Gd11: a pH responsive contrast agent conjugated to the G5-PAMAM dendrimer [61]

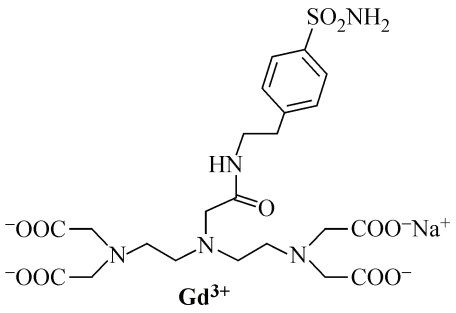


Fig. 20. Sulfonamide-substituted Gd-DTPA complex that shows high binding affinity towards carbonic anhydrase [49]

slow rotation of the agent, resulting in the enhanced overall relaxivity. The responsiveness of the relaxivity to pH for the dendrimer conjugate increased by more than a factor of 2 [61].

Enzyme responsive contrast agents are useful for measuring the enzyme activity as well as for their localization in the body. The response of contrast agents to specific enzymes is related to the mechanism of their interaction. Anelli *et al.* synthesized an enzyme responsive contrast agent (Gd-DTPA derivative) containing an arylsulfonamide moiety (Fig. 20). The agent was found to be a good inhibitor of carbonic anhydrase, a ubiquitous zinc enzyme which serves to interconvert carbon dioxide and bicarbonate to maintain the acid-base balance in blood and other tissues. It also helps in the transport of carbon dioxide out of tissues. The agent strongly interacts with the enzyme, resulting in an increased correlation time of the resultant adduct. The relaxivity of the bound form is about 5-fold greater than that of the free complex (27 $mM^{-1}s^{-1}$ vs. 5 $mM^{-1}s^{-1}$ at 20 MHz). However, this complex did not prove to be suitable as a blood-pool contrast agent due to a small quantity of carbonic anhydrase displayed on the surface of red blood cells.

In another approach, Gd^{3+} complexes serve as substrates for specific enzymes. Such enzyme-responsive contrast agents are converted to the compounds of high relaxivity via enzyme-catalyzed reactions. A Gd-DTPA derivative bearing a phosphoric ester shown in Fig. 21, acts in the same way. The hydrophobic biaryl group can interact strongly with hydrophobic binding sites of HSA. However, the presence of phosphate as a masking group to HSA reduces the affinity of the complex towards HSA. The hydrophilic phosphate group acts as a substrate for the enzyme alkaline phosphatase. In the presence of this enzyme, upon hydrolysis the phosphate group is cleaved, exposing a hydrophobic moiety for the interaction with HSA. Upon HSA association with the complex, relaxivity enhancement occurs due to the lengthening of the molecular reorientational time.

Similarly, a tris-lysine containing a ligand of a contrast agent can be cleaved by thrombin-activatable fibrinolysis inhibitor (TAFI) or carboxypeptidase, a plasma glycoprotein synthesized in the liver. Upon the cleavage of the tris-lysine peptide side chain, groups with high HSA affinity (diphenylalanine or 3,5-diiodotyrosine) are exposed (Fig. 22). Due to the enzymic action, a significant increase in the relaxivity of both diphenylalanine and diiodotyrosine derivatives is observed [13, 33, 49, 62].

Meade and coworkers demonstrated that the enzyme activity also influences the hydration state of the metal ion. They reported an analog of DOTA, i.e. galactopyranosyl substituted 1-hydroxyethyl-1,4,7,10-tetraazacyclododecane-4,7,10-triacetic acid (Fig. 23, R = H). Upon the coordination of this

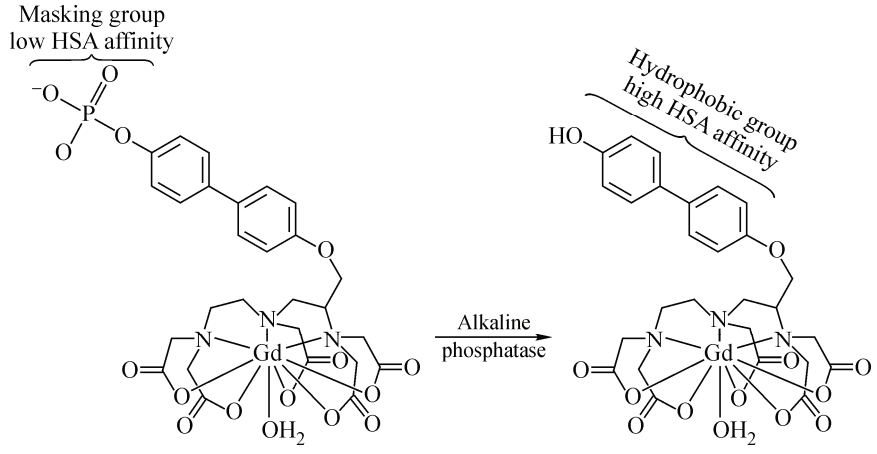


Fig. 21. Activation of a Gd-DTPA derivative by the alkaline phosphatase [13]

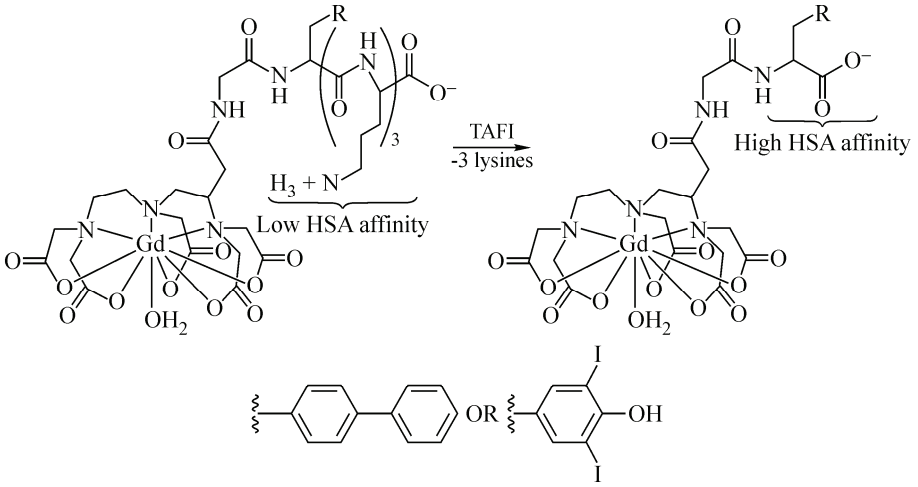


Fig. 22. Cleavage of a tris-lysine peptide side chain by TAFI in a Gd-DTPA derivative [62]

ligand with Gd^{3+}, the Gd(DO3A-gal) complex is formed in which all nine coordination sites of the metal ion are occupied. The galactose moiety blocks the ninth coordination site and thus prevents the binding of a water molecule to the metal ion in the first coordination sphere. The enzymatic action of β-galactosidase causes the cleavage of the sugar moiety, creating a free coordination site and thus ensuring the access of a water molecule to the Gd^{3+} ion. It was observed that a change in the hydration number from 0 to 1 due to the enzymatic cleavage of the galactose moiety resulted in a 20 % relaxivity enhancement of the Gd^{3+} complex. Gd(DO3A-gal) has been used for *in vivo* visualization of gene expression [62].

Metal ion responsive contrast agents. Some metal ions, such as iron and calcium, are very useful for the human body, while some others, such as lead, cadmium, and mercury, are extremely harmful. Variation in the concentration of these metal ions in the body causes serious diseases. Copper and iron deficiency causes anemia, while liver cirrhosis has been associated with copper excess in the body. Iron excess causes thalassemia and siderosis. Therefore, the determination of the concentration of metal ions in the body is very important for the diagnosis of various diseases. For this purpose, contrast agents sensitive to the metal ion concentration in the body have been developed [13]. The introduction of metal ions into paramagnetic complexes changes their relaxivity. Contrast agents responsive to calcium, zinc, copper, and iron have been designed. A Ca^{2+} sensitive contrast agent (Gd-DOPTA) reported in the literature is shown in Fig. 24. A Ca^{2+} ion is used to couple two Gd^{3+} DO3A moieties on the central BAPTA unit. The introduction of the Ca^{2+} ion into this complex results in a shift of four carboxylates (two for each Gd^{3+} chelate) from the Gd^{3+} ion to the Ca^{2+} ion. This produces coordination sites for four water molecules in the complex (two on each Gd^{3+} chelate) in the inner

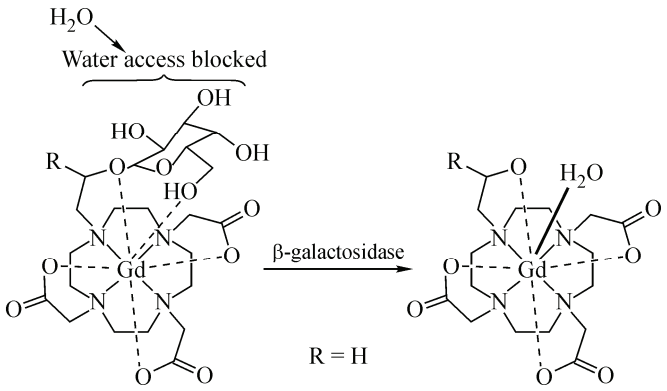


Fig. 23. Activation of a Gd^{3+}-based MRI contrast agent by the enzyme β-galactosidase (R = H) [62]

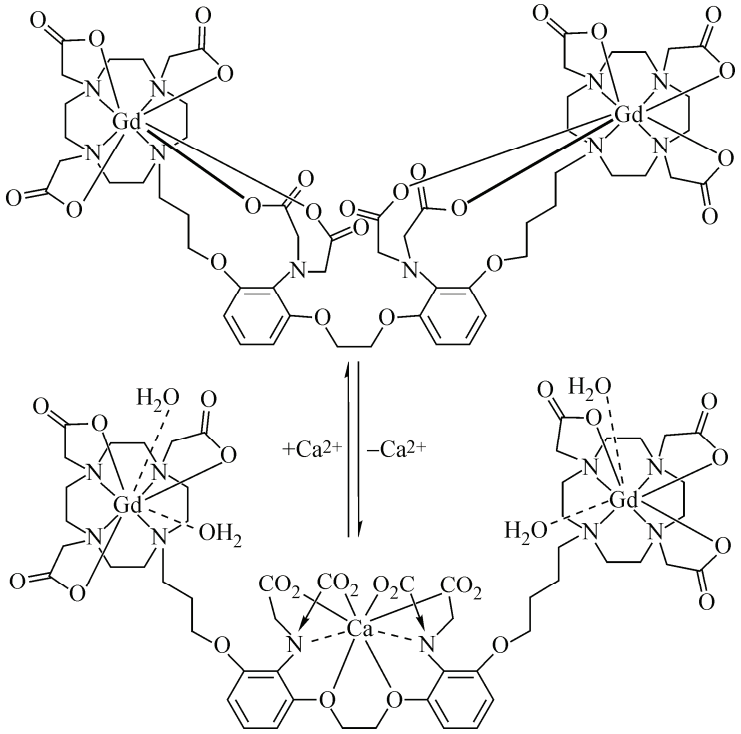


Fig. 24. Activation of a calcium-sensitive MRI contrast agent [29]

sphere. The calcium binding causes a considerable increase in the relaxivity of the complex (from 3.26 $mM^{-1}s^{-1}$ to 5.76 $mM^{-1}s^{-1}$ at 500 MHz and 25°C) [29].

Dhingra *et al.* have synthesized a monomeric calcium-sensitive contrast agent: Gd-DOPTRA (Fig. 25). The agent shows a ~100 % increase in its relaxivity (from 3.5 $mM^{-1}s^{-1}$ to 6.9 $mM^{-1}s^{-1}$ at 400 MHz) upon the addition of Ca^{2+} with a good selectivity to Ca^{2+} even in the presence of Mg^{2+} and Zn^{2+}. The *q* value was calculated from luminescence life measurements on Eu-DOPTRA. It was found to be 0.17 in the absence of Ca^{2+}, while upon the addition of Ca^{2+}, its value increased significantly and was recorded to be 0.88. It was concluded that an increase in the hydration number was responsible for the relaxivity enhancement. The relaxivity studies carried out in physiological fluids, such as the artificial extracellular matrix and the artificial cerebrospinal fluid, proved the agent to be suitable for *in vivo* measurements [63].

Hanaoka *et al.* reported the GdDTPA-bisBPEN complex as a Zn^{2+}-responsive MRI contrast agent. This complex is based on the DTPA ligand bifunctionalized with *N*,*N*-bis-(2-pyridyl-methyl) ethylene diamine (BPEN) (Fig. 26). The agent showed high selectivity for Zn^{2+} against Ca^{2+} and Mg^{2+}. It exhibited characteristic changes in the relaxivity for different concentrations of Zn^{2+}. In the absence

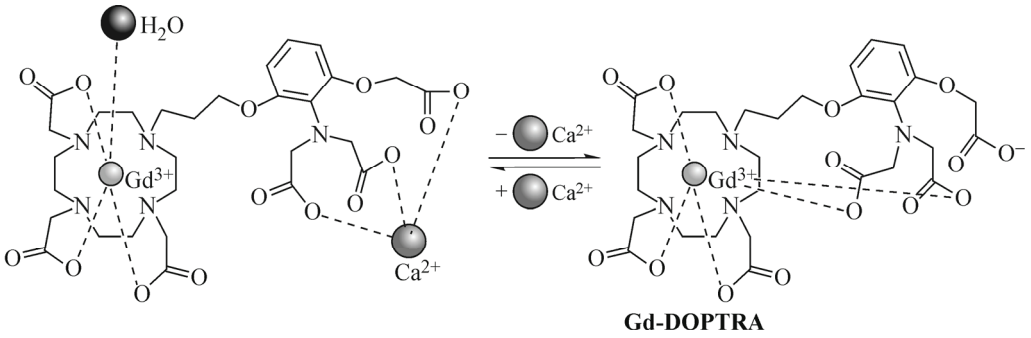


Fig. 25. Equilibrium reaction of Gd-DOPTRA with Ca^{2+} [63]

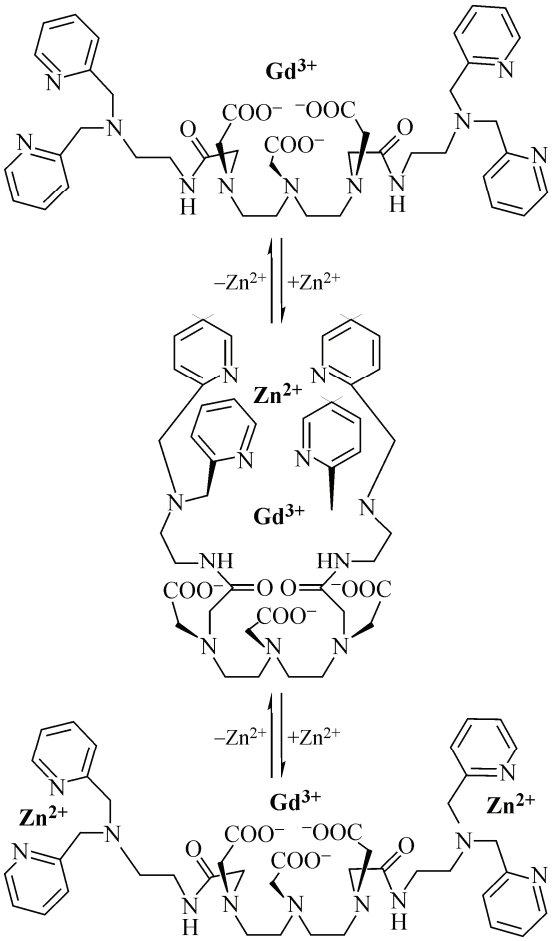


Fig. 26. Binding modes of the GdDTPA-bisBPEN complex with Zn^{2+} change according to the zinc concentration [64]

of Zn^{2+}, the complex has 6.06 $mM^{-1}s^{-1}$ relaxivity (300 MHz, 25 °C). However, by the addition of 1 equivalent of Zn^{2+} to the solution of this complex (1:1, Zn^{2+}:GdDTPA-bisBPEN), the relaxivity drops to 3.98 $mM^{-1}s^{-1}$ due to the displacement of a water molecule bound to the Gd^{3+} ion. The original relaxivity (6.06 $mM^{-1}s^{-1}$) of the complex is restored when another mole of Zn^{2+} (2 equivalents) is added to the solution of the complex. An increase in the Zn^{2+} concentration (2:1, Zn^{2+}:GdDTPA-bisBPEN) restores the same hydration number (q = 1) of the complex as it was in the absence of Zn^{2+} [64].

Esqueda and coworkers synthesized GdDOTA-diBPEN as another Zn^{2+} sensitive contrast agent (Fig. 27). The agent was found to have a strong affinity for Zn^{2+} and was suitable for the detection of its small quantity (30 μM of Zn^{2+}) in the presence of HSA by MRI *in vitro*. In the absence of Zn^{2+}, it binds weakly to HSA due to nonspecific electrostatic interactions. However, a ternary complex of GdDOTA-diBPEN and Zn^{2+} (1:2) shows a strong binding affinity for HSA. The relaxivity of the complex (GdDOTA-diBPEN) was reported to be 5.0 ± ± 0.1 $mM^{-1}s^{-1}$(37 °C, pH 7.6, 23 MHz). However, the addition of 2 equivalents of Zn^{2+} raised it to about 6.0 ± 0.1 $mM^{-1}s^{-1}$, which confirmed the formation of a 1:2 (Gd:Zn) complex. A ~165 % increase in the relaxivity of the ternary complex (GdDOTA-diBPEN-Zn) was observed upon its binding to HSA. The relaxivity increased from 6.6 ± 0.1 $mM^{-1}s^{-1}$ to 17.4 ± 0.5 $mM^{-1}s^{-1}$ [65].

A new class of copper-sensitive contrast agents called the copper-gad (CG) family was reported by Que and his coworkers. They coupled various thioether-based copper receptors to a Gd^{3+}-DO3A moiety via a 2,6-dimethylpyridine linker switch. (Fig. 28, *a*). The copper-binding groups contain different donor atoms (N, S, O) and denticities (3, 4, 5). These agents are sensitive to both Cu^{+} as well as Cu^{2+} ions. In the absence of copper, the pyridyl linker covers the DO3A unit and therefore, restricts the inner-sphere access of water ligands to the Gd^{3+} ion. It causes a decrease in the relaxivity. Upon the addition of copper ions, they bind to thioether-based copper receptors, therefore reduce the steric bulk around the Gd^{3+} center and it is exposed to the inner-sphere water access, resulting in relaxivity enhancement (Fig. 28, *b*). The addition of even 1 equivalent of copper ions caused a significant increase (from 92 % to 360 %) in the relaxivity of all the members of this class. The largest increase was observed for CG2 and CG3 whose relaxivities increased from 1.5 $mM^{-1}s^{-1}$ to 6.9 $mM^{-1}s^{-1}$ (at 37 °C, 60 MHz). Further the copper-induced relaxivity enhancement of these agents remained unaffected in the presence of Zn^{2+} excess. These are suitable agents for the sensitive detection of copper through MRI [66].

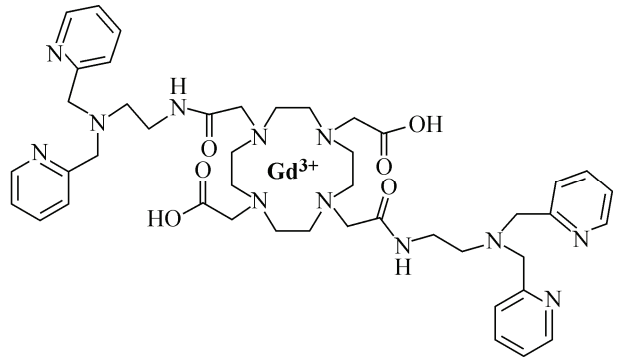


Fig. 27. Molecular structure of GdDOTA-diBPEN: a Zn^{2+}-sensitive contrast agent [65]

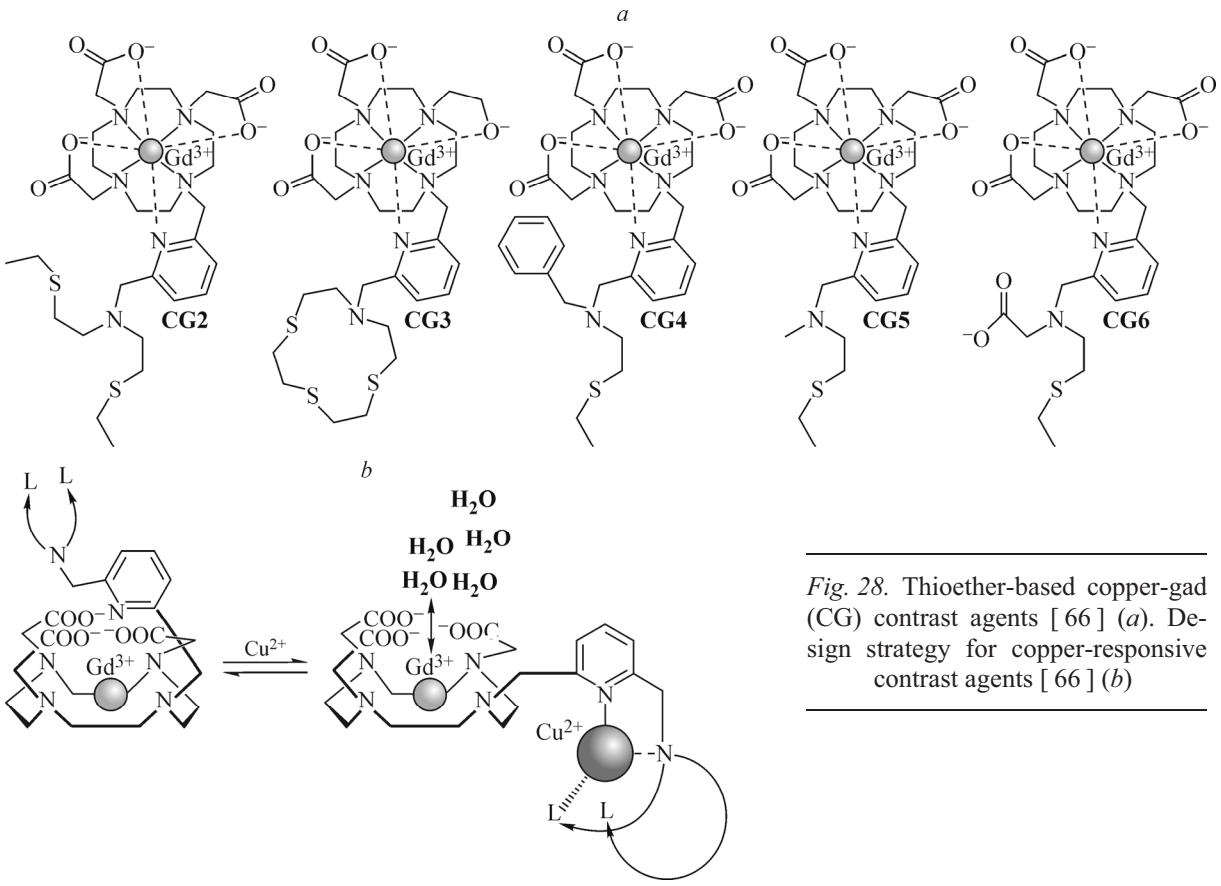


Fig. 28. Thioether-based copper-gad (CG) contrast agents [66] (*a*). Design strategy for copper-responsive contrast agents [66] (*b*)

Gd-PhenHDO3A is an agent whose relaxivity depends on the iron concentration. Gd-PhenHDO3A units gather around the Fe^{2+} ion to form another complex of a larger molecular weight (Fig. 29). In this example, the 5,6-dihydropenathroline subunit serves as a bidentate ligand for the chelation of both Fe^{2+} and Gd^{3+}. This causes a slow molecular tumbling and a prolonged rotational correlation time of the final complex. As a result of a slow molecular tumbling rate, the relaxivity is increased from 5.1 $mM^{-1}s^{-1}$ to 12.5 $mM^{-1}s^{-1}$ (about 145 % increase) [13].

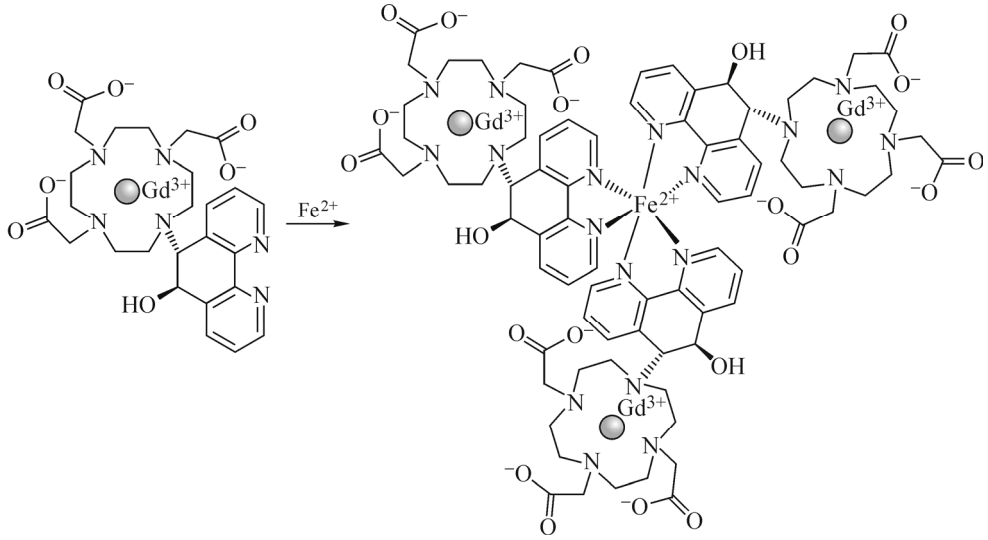


Fig. 29. Self-assembly of Gd-PhenHDO3A around Fe^{2+} resulting in relaxivity enhancement [13]

pO_2-Responsive contrast agents are used for the measurement of a partial oxygen pressure. The partial oxygen pressure is an important parameter in living systems, therefore its variation can cause several pathologies such as strokes and tumors. pO_2-Responsive contrast agents are developed by the use of metal ions that can switch between two redox states. Aime *et al.* reported Mn^{2+}/Mn^{3+} complexes with 5,10,15,20-tetrakis-(*p*-sulfonatophenyl) porphinate (tpps) as the pO_2-responsive contrast agents. Similarly, Eu^{2+}/Eu^{3+} complexes can act as the pO_2-responsive contrast agents. The relaxivity of these complexes depends on the partial oxygen pressure [67].

(5) CEST and PARACEST MRI contrast agents. As mentioned earlier, the conventional contrast agents currently in clinical use work by shortening the longitudinal or transverse relaxation times of protons near them. Although these contrast agents are very useful for the diagnosis of a number of pathologies like cancers, multiple sclerosis, and cartilage disease, still they have a few limitations. For example, MRI examinations performed with such contrast agents depend on the acquisition of precontrast images to help match and recognize the enhanced region. Therefore, a large agent dose is needed to produce clear contrast. Similarly, the measured effect of contrast agents responsive to the state of their environment (such as pH and temperature) depends on both the environment and the target concentration, which is usually unknown *in vivo.* This results in the complexity of quantification of the concerned environmental parameter. Furthermore, current targeted contrast agents work for the imaging of only one target site per MRI examination. In order to overcome these limitations of the conventional contrast agents, a new class of contrast agents called chemical exchange saturation transfer (CEST) agents has been developed. As the name (CEST) indicates, in this process, the chemical exchange of a nucleus during an NMR experiment occurs from one site to another chemically different site. In early days, it was also named as saturation transfer or magnetization transfer (MT). These agents generate contrast in a different way. They selectively decrease the magnetization of the water proton signal, with minimum effects on its longitudinal relaxation rate. Therefore, the molecules containing exchangeable protons (such as —OH and —NH) and MT-NMR are used to generate contrast [68, 69]. For a CEST agent, the exchange rates between the labile protons and the bulk water must be less than the difference in frequency ($\Delta\omega$) between the respective chemical environments, i.e.

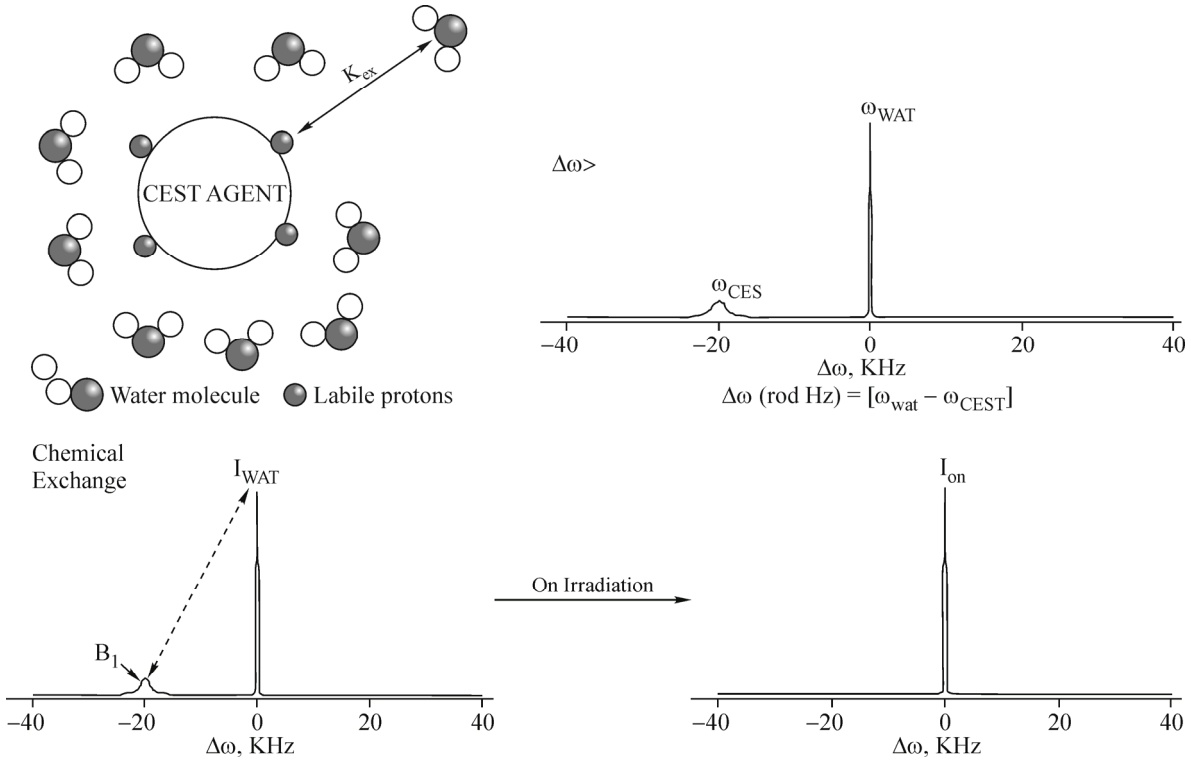


Fig. 30. Schematic representation of a CEST process [70]

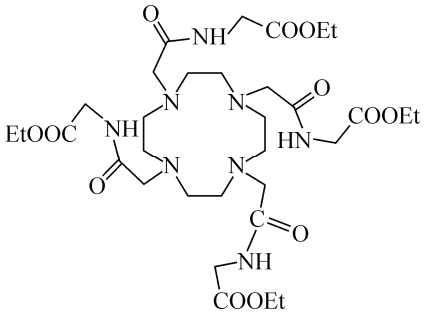


Fig. 31. Molecular structure of 1,4,7,10-tetraazacyclododecane tetrakis (ethyl-acetamidoacetate) [72]

$\Delta\omega \geq K_{ex}$. When this condition is achieved, the resonance of the mobile protons of the CEST agent can be selectively saturated using a specific radio frequency B_1, and then the chemical exchange process transfers the saturated magnetization from the mobile protons of the CEST agent to the bulk water molecules. Consequently, the signal intensity of these water molecules drops [70]. As $\Delta\omega$ increases with the field strength, the overall relationship between $\Delta\omega$ and K_{ex} will depend on the field strength of the MR experiment. The specificity of the CEST effect is improved by a larger $\Delta\omega$ value because for many tissues the magnetic field inhomogeneity (which causes broadening to their resonances) can exceed 2 ppm [71].

Since the achievable saturation transfer (ST) value is correlated to K_{ex}, it is expected that paramagnetic complexes, exhibiting high $\Delta\omega$ values for the exchanging proton resonance, can lead to the enhanced efficacy of CEST agents. These paramagnetic chelates called PARACEST agents consist of a specific Ln^{3+} complex with a coordinated water molecule, undergoing a very slow exchange with the bulk water and with extremely high $\Delta\omega$ values. Zhang and his coworkers demonstrated a good ST effect by the irradiation of metal-bound water protons of novel Eu^{3+}-based chelate (1,4,7,10-tetraazacyclododecane tetrakis (ethyl-acetamidoacetate)) resonating at 50 ppm downfield from the bulk water [72].

The magnitude of the PARACEST effect depends on many factors such as the lifetime of bound water, agent concentration, hyperfine shift, T_1/T_2 values, strength of the applied RF irradiation, and frequency offset. For two pools of protons (pool A consists of bulk water and pool B consists of water bound to the chelated lanthanide ion) with different NMR chemical shifts, the modified Bloch equations are used to describe the magnitude of the PARACEST effect. A mathematical relationship between the net magnetization of bulk water (M_Z^a) (after a long presaturation pulse) at the exchanging site and the PARACEST agent concentration is described by Eq. 13.

$$\frac{M_Z^a}{M_0^a} = \frac{\tau_a}{\tau_a + T_{1a}} = \left(1 + \frac{Cq}{55.5}\frac{T_{1a}}{\tau_m}\right)^{-1}. \quad (13)$$

Whereas in this equation C shows the agent concentration; q is the number of bound water molecules per PARACEST complex; τ_m indicates the lifetime of bound water; τ_a is the lifetime of a proton in pool A, and T_{1a} represents the spin-lattice relaxation time of bulk water. The relationship also explains the key roles of small τ_a and large T_{1a} values in the minimization of M_Z^a / M_0^a [73].

CONCLUSIONS

Gd^{3+}-based contrast agents have proved to be very useful in the imaging of various pathologies via the use of the MRI technique. Their application ensures clear contrast between diseased and normal tissue. These agents must be safe at their effective dose. In order to prevent any chronic effects, they must not dissociate to a considerable extent *in vivo*. Less stable contrast agents are harmful for the *in vivo* use since their transmetalation results in the release of free gadolinium. Due to a high zinc concentration (55—125 μmol/l) in the blood, it displaces Gd^{3+} within the chelate, liberating free gadolinium that then deposits in tissues. In the case of less stable contrast agents, released free gadolinium is retained in tissues for a long time. In patients with advanced renal impairment, the deposition of free gadolinium in tissues leads to NSF. Therefore, the use of highly stable contrast agents is extremely important for diagnostic purposes. The stability of contrast agents depends on their chemical structure. Ionic macrocyclic chelates have been reported to be the most stable because Gd^{3+} is strongly bound in pre-organized rigid rings. Gd-DOTA (Gadoterate), an ionic macrocyclic chelate has been reported to be the most stable contrast agent. No cases of NSF have been reported using stable macrocyclic gadolinium contrast agents. Non-ionic linear chelates do not bind strongly to Gd^{3+} and are therefore unsta-

ble. Among such agents, Gd-DTPA-BMEA (Gadoversetamide) and Gd-DTPA-BMA (Gadodiamide) are known to be the least stable [74].

Current research around the world mainly focuses on the synthesis of novel contrast agents with high relaxivity and target specificity. We have explained this idea with a few suitable examples in this article. However, the demands for stability and targeting are more important than the enhanced relaxivity. The use of Gd^{3+}-based contrast agents should be avoided in patients with kidney failure, unless the diagnostic data is necessary and cannot be obtained with non-contrast enhanced MRI. The majority of responsive Gd^{3+} contrast agents reported in the literature did not prove to be useful for *in vivo* use. Future work is to be focused on the synthesis of efficient, high-relaxivity, target-specific contrast agents with fewer side effects and suitable for *in vivo* use. For the synthesis of stable and targe-specific contrast agents, new types of ligand architectures need to be examined. Coupling of the existing low molecular weight complexes with high molecular weight HAS-like other molecules can result in their enhanced relaxivity. The use of lanthanide ion complexes as PARACEST agents has lead to the development of a new class of contrast agents with increased $\Delta\omega$ and better properties than the conventional diamagnetic agents. There has recently been a growing interest in the synthesis of Gd^{3+} complexes based on high molecular weight porphyrin derivatives since they are expected to be efficient tumor targeting agents.

REFERENCES

1. *Lauffer R.B.* // Chem. Rev. – 1987. – **87**. – P. 901 – 27.
2. *Squire L.F., Novelline R.A.* Squire′s fundamentals of radiology (6th ed.). Harvard University Press, 2004. – P. 36.
3. *Rajan S.S.* MRI: a conceptual overview. – New York: Springer-Verlag, Inc, 1997. – P. 1.
4. *Wong W.S., Tsukuda J.S., Kortman K.E., Bradley W.G.* Practical Magnetic Resonance Imaging. A case study approach. – USA: Aspen publishers Inc., 1987. – P. 1.
5. *Ratnakar S.J., Alexander V.* // Eur. J. Inog. Chem. – 2005. – P. 3918 – 3927.
6. *Mettler F.A., Muroff L.R., Kulkarni M.V.* Magnetic Resonance Imaging and Spectroscopy. – New York: Churchill Livingstone, 1986. – P. 231.
7. *Gallez B., Swartz H.M.* // NMR in Biomedicine – 2004. – **17**. – P. 223 – 5.
8. *Caravan P.* // Chem. Soc. Rev. – 2006. – **35**. – P. 512 – 523.
9. *Raymond N.K., Pierre C.V.* // Bioconjugate Chem. – 2005. – **16**. – P. 3 – 8.
10. *Gries H.* // Topics in Current Chemistry. – 2002. – Vol. 221. – P. 1 – 24 (Contrast Agents I). – Berlin, Heidelberg: Springer-Verlag.
11. *Yan P.G., Robinson L., Hogg P.* // Radiography. – 2007. – **13**. – P. 5 – 19.
12. *Qin J., Laurent S., Jo S.Y., Roch A., Mikhaylova M., Bhujwalla M.Z., Muller N.R., Muhammed M.A.* // Adv. Mater. – 2007. – **19**. – P. 1874 – 1878.
13. *Jacques V., Desreux J.F.* // Topics in Current Chemistry. – 2002. – Vol. 221. – P. 123 – 164 (Contrast Agents 1). – Berlin, Heidelberg: Springer-Verlag.
14. *Sherry A.D., Caravan P., Lenkinski R.E.* // J. Magn. Res. Imag. – 2009. – **30**. – P. 1240 – 1248.
15. *Brucher E.* // Topics in Current Chemistry. – 2002. – Vol. 221. – P. 103 – 122 (Contrast Agents 1). – Berlin, Heidelberg: Springer-Verlag.
16. *Strijkers G.J., Mulder W.J.M., Tilborg G.A.F.V., Nicolay K.* // MRI Contrast Agents: Curr. Stat. Future Perspect. Anti-cancer Agents Med. Chem. – 2007. – **7**. – P. 291 – 305.
17. *Toth E., Helm L., Merbach A.E.* // Topics Curr. Chem. – 2002. – Vol. 221. – P. 61 – 101 (Contrast Agents 1). – Berlin, Heidelberg: Springer-Verlag.
18. *Borel A., Bean J.F., Clarkson R.B., Helm L., Moriggi L., Sherry A.D., Woods M.* // Chem. Eur. J. – 2008. – **14**. – P. 2658 – 2667.
19. *Werner E.J., Datta A., Jocher C.J., Raymond K.N.* // Angew. Chem. Int. Ed. – 2008. – **47**. – P. 8568 – 8580.
20. *Kumar K., Chang C.A., Francesconi L.C., Dischino D.D., Malley M.F., Gougoutas J.Z., Tweedle M.F.* // Inorg. Chem. – 1994. – **33**. – P. 3567 – 3575.
21. *Aime S., Cabella C., Colombatto S., Crich S.G., Gianolio E., Maggioni F.* // J. Magn. Res. Imag. – 2002. – **16**. – P. 394 – 406.
22. *Jacques V., Dumas S., Sun W.C., Troughton J.S., Greenfield M.T., Carvan P.* // Investigat. Radiol. – 2010. – **45**. – P. 613 – 624.
23. *Lowe M.P.* // Aust. J. Chem. – 2002. – **55**. – P. 551 – 556.

24. *Geraldes C.F.C.* // Contrast Media Mol. Imaging. – 2009. – **4**. – P. 1 – 23.
25. *Weinmann H.J., Ebert W., Misselwitz B., Willich H.S.* // Eur. J. Radiol. – 2003. – **46**. – P. 33 – 44.
26. *Granier T., Langlois d'Estaintot B., Gallois B., Chevalier J.M., Précigoux G., Santambrogio P., Arosio P.* // J. Biol. Inorg. Chem. – 2003. – **8**, N 1-2. – P. 105 – 111.
27. *Carvan P., Cloutier N.J., Greenfield M.T., McDermid S.A., Dunham S.U., Bulte J.W.M., Amedio J.C., Looby R.J., Supkowski R.M., Horrocks W.D., McMurry T.J., Lauffer R.B.* // J. Amer. Chem. Soc. – 2002. – **124**. – P. 3152 – 3162.
28. *Haen C.D., Anelli P.L., Lorusso V., Morisetti A., Maggioni F., Zheng J., Uggeri F., Cavagna F.M.* // Investigat. Radiol. – 2006. – **41**. – P. 279 – 291.
29. *Woods M., Shanrong Z., Sherry D.* // Curr. Med. Chem.-Immun., Endoc. & Metab. Agents. – 2004. – **4**. – P. 349 – 369.
30. *Aime S., Botta M., Fasano M., Terreno E.* // Chem. Soc. Rev. – 1998. – **27**. – P. 19 – 29.
31. *Chan K.W.Y., Wong W.T.* // Coord. Chem. Rev. – 2007. – **251**. – P. 2428 – 2451.
32. *Caravan P., Ellison J.J., McMurry T.J., Lauffer R.B.* // Chem. Rev. – 1999. – **99**. – P. 2293 – 2352.
33. *Aime S., Botta M., Terreno E.* // Adv. Inorg. Chem. – 2005. – **57**. – P. 173 – 237.
34. *Wong W.-T., Li C.* Paramagnetic complexes with pendant crown compounds showing improved targeting-specificity as MRI contrast agents. – 2009/04/23, US 2009/0104124 A1.
35. *Aime S., Botta M., Fasano M., Crich S.G.* // Coord. Chem. Rev. – 1999. – **185-186**. – P. 321 – 333.
36. *Hermann P., Kotek J., Kubicek V., Lukes I.* // Dalton Trans. – 2008. – P. 3027 – 3047.
37. *Laus S., Ruloff R., Toth E., Merbach A.E.* // Chem. Eur. J. – 2003. – **9**. – P. 3555 – 3566.
38. *Micskei K., Helm L., Brucher E., Merbach A.E.* // Inorg. Chem. – 1993. – **32**. – P. 3844 – 3850.
39. *Bloembergen N.* // J. Chem. Phys. – 1957. – **27**. – P. 572 – 573.
40. *Carvan P., Astashkin A.V., Raitsimring A.M.* // Inorg. Chem. – 2003. – **42**. – P. 3972 – 3974.
41. *Zhang Z., Nair S.A., McMurry T.J.* // Current Med. Chem. – 2005. – **12**. – P. 751 – 778.
42. *Saini S.* // Principles, AJR. – 1991. – **156**. – P. 236 – 239.
43. *Bellin M.-F., Van Der Molen A.J.* // Eur. J. Radiol. – 2008. – **66**. – P. 160 – 167.
44. *Broome D.R.* // Eur. J. Radiol. – 2008. – **66**. – P. 230 – 234.
45. *Thomsen H.S.* // Eur. Radiol. – 2006. – **16**. – P. 2619 – 2621.
46. *Kuo P.H., Kanal E., Abu-Alfa A.K., Cowper S.E.* // Radiol. – 2007. – **242**. – P. 647 – 649.
47. *Clarkson R.B.* // Topics in Current Chemistry. – 2002. – **221**. – P. 201 – 235 (Contrast Agents 1). – Berlin, Heidelberg: Springer-Verlag.
48. *Knopp M.V., Tengg-Kobligk H.V., Floemer F., Schoenberg S.O.* // J. Magn. Res. Imag. – 1999. – **10**. – P. 314 – 316.
49. *Pereira G.A., Geraldes C.F.G.C.* // Ann. Magn. Reson. – 2007. – **6**. – P. 1 – 33.
50. *Misselwitz B., Schmitt-Willich H., Ebert W., Frenzel T., Schmitt-Willich H.* // Magn. Res. Mater. Phys., Biol. Med. – 2001. – **12**. – P. 128 – 134.
51. *Gaillard S., Kubiak C., Stolz C., Bonnemain B., Chassard D.* // Invest Radiol. – 2002. – **37**, N 4. – P. 161-6.
52. *Port M., Corot C., Rousseaux O., Raynal I., Devoldere L., Idee J.M., Dencausse A., Greneur S.L., Simonot C., Meyer D.* // Magma. – 2001. – **12**, N 2-3. – P. 121 – 7.
53. *Guoping Y., Renxi Z.* // Chin. Sci. Bull. – 2001. – **46**. – P. 1233 – 1237.
54. *Kobayashi H., Brechbiel M.W.* // Adv. Drug Deliv. Rev. – 2005. – **57**. – P. 2271 – 2286.
55. *Cavagna F.M., Dapra M., Castelli P.M., Maggioni F., Kirchin M.A.* // Eur. Radiol. – 1997. – Suppl. 5. – P. S222 – S224.
56. *Frullano L., Rohovec J., Peters J.A., Geraldes C.F.G.C.* // Top. Curr. Chem. – 2002. – **221**. – P. 25 – 50 (Contrast Agents I). – Berlin, Heidelberg: Springer-Verlag.
57. *Artemov D.* // J. Cellular Biochem. – 2003. – **90**. – P. 518 – 524.
58. *Frullano L., Rohovec J., Aime S., Maschmeyer T., Prata M.I., Pedroso de Lima J.J., Geraldes C.F.G.C., Peters J.A.* // Chem. Eur. J. – 2004. – **10**. – P. 5205 – 5217.
59. *Boutry S., Burtea C., Laurent S., Toubeau G., Elst L.V., Muller R.N.* // Magn. Res. Med. – 2005. – **53**. – P. 800 – 807.
60. *Hovland R., Glogard C., Aasen A.J., Klaveness J.* // J. Chem. Soc., Perkin Trans. – 2001. – **2**. – P. 929 – 933.
61. *Ali M.M., Woods M., Carvan P., Opina A.C.L., Spiller M., Fettinger J.C., Sherry A.D.* // Chem. Eur. J. – 2008. – **14**. – 7250 – 7258.
62. *Lowe M.P.* // Curr. Pharm. Biotechnol. – 2004. – **5**. – P. 519 – 528.
63. *Dhingra K., Maier M.E., Beyerlein M., Angelovski G., Logothetis N.K.* // Chem. Commun. – 2008. – **29**. – P. 3444 – 3446.

64. *Hanaoka K., Kikuchi K., Urano Y., Nagano T.* // Chem. Soc., Perkin Trans. – 2001. – **2**. – P. 1840 – 1843.
65. *Esqueda A.C., Lopez J.A., Andreu-de-Riquer G., Alvarado-Monzon J.C., Ratnakar J., Lubag A.J.M., Sherry A.D., De Leon-Rodriguez L.M.* // J. Amer. Chem. Soc. – 2009. – **131**. – P. 11387 – 11391.
66. *Que E.L., Gianolio E., Baker S.L., Wong A.P., Aime S., Chang C.J.* // J. Amer. Chem. Soc. – 2009. – **131**. – P. 8527 – 8536.
67. *Aime S., Botta M., Gianolio E., Terreno E.* // Angew. Chem. Int. Ed. – 2000. – **39**, N 4. – P. 747 – 750.
68. *Hancu I., Dixon W.T., Woods M., Vinogradov E., Sherry A.D.* // Acta Radiol. – 2010. – **8**. – P. 910 – 923.
69. *Woods M., Woessner D.E., Sherry A.D.* // Chem. Soc. Rev. – 2006. – **35**. – P. 500 – 511.
70. *Aime S., Crich S.G., Gianolio E., Giovenzana G.B., Tei L., Terreno E.* // Coord. Chem. Rev. – 2006. – **250**. – P. 1562 – 1579.
71. *Guivel-Scharen V., Sinnwell T., Wolff S.D., Balaban R.S.* // J. Magn. Res. – 1998. – **133**. – P. 36 – 45.
72. *Zhang S., Winter P., Wu K., Sherry A.D.* // J. Amer. Chem. Soc. – 2001. – **123**. – P. 1517 – 1518.
73. *Zhang S., Merritt M., Woessner D.E., Lenkinski R.E., Sherry A.D.* // Acc. Chem. Res. – 2001. – **36**. – P. 783 – 790.
74. *Morcos S.K.* // Eur. J. Radiol. – 2008. – **66**. – P. 175 – 179.