Metal organo phosphorous compounds for NMR analysis.


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The loss of detail in NMR spectra can limit the use of NMR analysis. Contrast agents have been employed in order to improve NMR imaging for non-invasive clinical diagnoses of mammalian hosts. The present invention relates to a class of compositions and a method for NMR imaging using NMR signal affecting amounts of a paramagnetic, diamagnetic or ferromagnetic metal ion chelated with an organo phosphorus compound.

The use of rare earth metal ions chelates of beta-diketones as transparent NMR shift reagents are described by Belager et al. in U.S. Patent 3,818,061. The patentees describe the novel chelates as useful NMR shift reagents because they not only solubilize the rare earth metal ion but additionally the beta-diketone ligand is substantially transparent or completely transparent to proton NMR analysis.

Compounds for affecting the relaxation time in NMR diagnostics is described by Gries et al. in European Patent Application 0 071 594. Phosphorus based chelated lanthanide metal ions are specifically disclosed by Gries et al.

Various bis-phosphinyl phosphinates are described as sequestrants by Knollmuller in U.S. Patent 3,534,125. The patentee also describes these phosphinates which can be chelated with rare earth metal ions. Budnick, U.S. Patent 4,116,980 and Carlson, U.S. Patent 3,477,953 describe various phosphate chelating compounds as well.

Anderegg, G. in Naturforsch., B: Anorg. Chem./ Org. Chem., 1977, 32B(6), 547-50 and abstracted in C. A. 86; 195-960 (1977) studied the stability constants and the formation of the 1:1 complexes of Mg²⁺, Ca²⁺, Ni²⁺, Cu²⁺ and Zn²⁺ with tris(dihydroxy-phosphinyl)methyl phosphine oxide pH-metrically at 20°C and ionic strength 0.1 (Me₄NCl).

NMR imaging has emerged in recent years as a superior technique for noninvasive clinical diagnosis of the heart, brain, kidney and other organs and tissues in mammalian hosts. In many instances, in order to obtain useful images, contrast enhancement is needed to delineate various aspects of the tissue especially normal as contrasted with abnormal tissue.

The prior art discloses that various techniques can be employed for affecting an NMR signal in a host, the most common of which is to introduce into the host a paramagnetic substance prior to NMR analysis or imaging. This is commonly achieved by employing polyvalent ions of a paramagnetic metal ion such as for example, iron, manganese, chromium, copper, nickel and metal ions of the lanthanide series. Gadolinium as a contrast agent for NMR has also been described by Callie’ et al. (AJNRA: 1041—1042, September/ October (1983)) and that gadolinium is especially useful in this respect since it is the rare earth element that possesses the highest paramagnetic moment, 10.8 Bohr magnetons. Although these ions of paramagnetic metal ions enhance NMR imaging, it has been reported that gadolinium is best used as a chelated ion to reduce its toxicity (Carr et al., The Lancet, March 3, 1984 pp.484–488). Additionally, Mendonca-Dias et al. (Seminars in Nuclear Medicine, Vol. XIII, No. 4 [October 1983 pp. 364—376) describe various paramagnetic contrast agents in nuclear magnetic resonance for medical imaging but caution against the use of copper, nickel and iron ions which have acute toxicities which are higher than manganese. The authors describe the long-term toxic effect of manganese (Manganese), as producing several neurologic and psychiatric disorders, which in the late stages resembles Parkinson’s disease. Lanthanide toxicity is also described by the authors and that a certain amount of precautions have to be employed when using metal ions from this group.

The authors disclose that toxicity is significantly reduced if the contrast enhancing metal ion ions are chelated or if they are selected so that only those that are flushed from the body rapidly are employed. If these ions are flushed too quickly from the body, their effectiveness in NMR analysis may be minimized or lost.

The contrast enhancing agents for medical NMR imaging are effective because the metal ion ions and their complexes may concentrate selectively in abnormal tissues, (Mendonca-Dias et al. vide supra) and the paramagnetic ions increase the relaxation rates of water protons at low concentration in the tissue. Chauncey et al., J. Nucl. Med., 1977; 18: 933—936 have also demonstrated the opposite in that radioactive Mn⁴⁺ accumulated in normal myocardial tissue while infarcted myocardial tissue had reduced levels. As noted above, the use of some of these metal ion ions is not without its difficulties.

Many of the chelating agents used for paramagnetic, diamagnetic and ferromagnetic ions are not completely effective in keeping the ions complexed when administered to mammalian hosts since the complexes, during metabolism, can be broken down such as for example, in the use of phosphorous chelating agents which are in some instances hydrolyzed by phosphate-hydrolyzing enzymes abundant in living tissues.

In accordance with the present invention to overcome these and other difficulties encountered in the prior art are overcome.

The present invention relates to a composition for affecting an NMR signal employing a complex of a paramagnetic metal ion, diamagnetic metal ion or ferromagnetic metal ion and an organo-phosphorous chelating ligand which is not readily hydrolyzed by phosphate-hydrolyzizing enzymes abundant in living tissues and which optionally enhances the NMR contrasting capability of the uncomplexed or nonchelated metal ion when such complex is administered to a host that is subsequently subjected to NMR analysis.

Furthermore, the present invention is useful for NMR analysis comprising an NMR signal affecting amount of a complex of a paramagnetic metal ion, diamagnetic metal ion and a ferromagnetic metal ion and an organophosphorous metal ion.
chelating ligand which is relatively nontoxic in mammalian hosts, it is not readily hydrolyzable by phosphate-hydrolyzing enzymes and will remain in a mammalian host relatively intact for a period of time sufficient to perform an NMR analysis on said host.

In addition, the complex concentrates selectively in abnormal tissues in a mammalian host to enhance the NMR analysis of such abnormal tissue and selectively in normal tissues which also contain abnormal tissue in a mammalian host to enhance the NMR analysis of the tissues.

More particularly, the present invention relates to the use of the complexes of:

a) a metal ion selected from metal ions having atomic number 21 to 29 inclusive, 42 to 44 inclusive and the lanthanides having atomic numbers 57 to 70 inclusive, and

b) an organophosphorous metal ion chelating ligand comprising a phosphonylorgano phosphinite to form complexes of the formula:

\[
\begin{align*}
&\begin{array}{c}
O \\
&+P-R+ P-O^-
\end{array} \\
&\begin{array}{c}
O \\
&+P-R+ P-O^-
\end{array}
\end{align*}
\begin{align*}
&a(n_{n+3}^\text{+})+v \\
&\begin{array}{c}
R_1 \\
&+P-R+
\end{array} \\
&\begin{array}{c}
O \\
&+P-R+
\end{array}
\end{align*}
\]

wherein

\(n=2\) to about 100;
\(n_{n+3}\) is the number of negative charges in

\[
\begin{align*}
&O \\
&+\begin{array}{c}
P-R+
\end{array} \\
&\begin{array}{c}
R_1 \\
&+P-R+
\end{array}
\end{align*}
\]

groups;

\(a=1, 2\) or 3;

\(M=\) a metal ion described hereinabove;

\(v=\) the valence of \(M\);

\(R_1=O^-\) or an organophosphenate comprising

\(\begin{array}{c}
P-R+
\end{array}\)

containing from 1 to 3 carbon atoms; cyclic hydrocarbons having from 3 to about 10 carbon atoms, said cyclic hydrocarbon being saturated or unsaturated and including fused ring cyclic hydrocarbons; heterocyclic hydrocarbons having from 1 to 2 heterocyclic nitrogen, phosphorous, sulfur or oxygen atoms in said heterocyclic ring, said heterocyclic hydrocarbon being saturated or unsaturated, in a method for NMR analysis.

The NMR signal affecting amount of the complex is any amount of complex that will alter the spin-lattice, spin-spin or spin-echo relaxation times of an NMR signal. This alteration is effected in a manner in order to enhance the signals received from the specimen under analysis either by reducing the aforementioned relaxation times or by increasing them with respect to an area of the host or the host per se which has had the complex administered to it. In another embodiment, the NMR signal affecting amount of the complex is that amount which in addition to changing the relaxation times of the NMR signals in the host, will also change such relaxation times sufficiently so that sharper lines of definition or higher contrast is obtained between those parts of the host that have and have not been administered the complex.

The preferred metal ions comprise the metal ions from the lanthanide group of the Periodic Table of the Elements and comprise those metal ions having atomic numbers 57—70 inclusive and those metal ions having atomic numbers 21—29 inclusive and 42—44 inclusive especially copper, manganese, iron and chromium.

The especially preferred examples of the present invention comprises water soluble anionic coordination-complexes, especially the water soluble anionic coordination-complexes of the lanthanide metal ions.

The host to which the complex may be administered may be animate or inanimate. Either living or non-living tissue of an animate host may have the complex administered to it, although one of the principal objectives of the present invention is to provide a complex that is employed in a living host which is subject to NMR analysis and especially NMR imaging techniques.

A preferred complex is one of the above formulae where \(R\) is methyl.

An especially preferred complex has the formula:

\[
\begin{align*}
&O \\
&+\begin{array}{c}
P-R+
\end{array} \\
&\begin{array}{c}
R_1 \\
&+P-R+
\end{array}
\end{align*}
\]

where \(a=1, 2\) or 3.

The organophosphorous metal ion chelating ligand comprises a phosphonylloweralkyl phosphinate derived from phosphorous compounds comprising either bis - dihydroxophosphonyllower-alkyl - phosphinic acid or tris - dihydroxophosphonylloweralkyl - phosphine oxide. In a preferred embodiment, the organophosphorous metal ion chelating ligand is not readily hydrolyzable by phosphate-hydrolyzing enzymes. The alkyl moiety includes straight or branch chain alkyl radicals where the straight chain alkyl radicals are preferred, in which the alkyl moiety has from one to three carbon atoms. The especially preferred lower alkyl moiety has one carbon atom. Specific phosphorous compounds suitable in this regard are bis - dihydroxophosphonylmethyl - phosphinic acid and tris - dihydroxophosphonylmethyl - phosphine oxide.

according to the method disclosed by Knoll-
müller in U.S. Patent 3,534,125 incorporated
herein by reference. This synthesis comprises
converting bis(hydroxymethyl) phosphonic acid
by means of thionyl chloride to bis(chloromethyl)
phosphonic acid chloride. The bis(chloromethyl)
phosphonic acid chloride is reacted with an
organic alcohol or hydrocarboxyl hydroxyl
compound to produce an intermediate chloro-
methyl ester. The chloromethyl ester is then
reacted with a tri-hydroxycarbonyl phoshphite by the
Michaelis-Arbusov reaction. The ester obtained
from this latter reaction is then heated with a
strong acid such as hydrochloric acid to hydrolyze
it to the phosphonic acid phosphorous containing
compounds employed according to the present
invention.

Upon obtaining the phosphonic acid phos-
phorous compound, it may be neutralized with an
appropriate base such as an alkaline earth metal
ion or alkali metal ion based. The salt of the
phosphonic acid compound is then reacted with a
metal ion salt (i.e. a paramagnetic or ferro-
magnetic metal ion salt) in metathesis reaction
whereby the phosphonic acid compound will
complex with the metal ion.

The complexes are then administered to a host
and the host, thus treated is subjected to NMR
analysis. This analysis is best understood by a
brief explanation of NMR phenomenon.

Nuclear magnetic resonance phenomenon
occurs in atomic nuclei having a non-zero nuclear
spin. Due to its spin each nucleus exhibits a
magnetic moment, so that, when a sample
composed of such nuclei is placed in a static,
homogeneous magnetic field, $B_0$, a greater
number of nuclear magnetic moments align with
the field to produce a net macroscopic magne-
tization (M) also referred to as longitudinal magne-
tization) in the direction of the field. Under
the influence of the magnetic field $B_0$, magnetization
M precesses about the axis of the field at a
frequency which is dependent on the strength of
the applied magnetic field and on the charac-
teristics of the nuclei. The angular precession
frequency, $\omega$, also referred to as the Larmor
frequency, is given by the Larmor equation $\omega = \gamma B_0$, $\gamma$ in which $\gamma$ is the gyromagnetic ratio which is constant for each NMR isotope and wherein $B_0$ is the magnetic field acting upon the nuclear spins.
It will be thus apparent that the resonant
frequency is dependent on the strength of the
magnetic field in which the sample is positioned.
The orientation of magnetization $M$, normally
directed along the magnetic field $B_0$, may be
perturbed by the application of a magnetic field
oscillating at the Larmor frequency. Typically,
such a magnetic field, designated $B_1$, is applied in
a plane orthogonal to the direction of the static
magnetic field by means of a radio frequency (RF)
pulse through coils connected to a radio-fre-
quency-transmitting apparatus. The effect of field
$B_1$ is to rotate magnetization $M$ about the
direction of the $B_0$ field. This may be best
visualized if the motion of magnetization $M$ due to
the application of RF pulses is considered in a
Cartesian coordinate system which rotates at a
frequency equal to the resonant frequency about the main magnetic field $B_0$ in
the same direction in which the magnetization $M$
precesses (i.e., the rotating frame). In this case,
the positive direction of the $Z$-axis, is typically
chosen to be directed along $B_0$ which, in the
rotating frame $Z$, is designated $Z'$ to distinguish it
from the fixed-coordinate system. Similarly, the
$X$- and $Y$-axes are designated $X'$ and $Y'$. Bearing
this in mind, the effect of an RF pulse, then, is to
rotate magnetization $M$, for example, from its
direction along the positive $Z'$ axis toward the
transverse plane defined by the $X'$ and $Y'$ axes.
An RF pulse having either sufficient magnitude or
duration to rotate magnetization $M$ into the
transverse plane (i.e., $90^\circ$ from the direction of the
$B_0$ field) is conveniently referred to as a $90^\circ$ RF
pulse. Similarly, proper selection of either
magnitude and/or duration of an RF pulse will
cause magnetization $M$ to change direction from
the positive $Z'$ axis to the negative $Z'$ axis. This
kind of an RF pulse is referred to as a $180^\circ$ RF
pulse, or for obvious reasons, as an inverting
pulse. It should be noted that a $90^\circ$ or a $180^\circ$ RF
pulse will rotate magnetization $M$ through the
corresponding number of degrees from any initial
direction of magnetization $M$. It should be further
noted that an NMR signal will only be observed if
magnetization $M$ has a net transverse component
in the transverse plane (perpendicular to $B_0$).
Assuming an initial orientation of magnetization
$M$ in the direction of the $B_0$ field, a $90^\circ$ RF pulse
produces maximum net transverse magnetization
in the transverse plane since all of magnetization
$M$ is in that plane, while a $180^\circ$ RF pulse does
not produce any transverse magnetization. The $180^\circ$
RF pulses are frequently utilized to produce NMR
spin-echo signals.

RF pulses may be selective or nonselective.
Selective pulses are typically modulated to have a
predetermined frequency content so as to excite
nuclear spins situated in preselected regions of
the sample having precession frequencies as
predicted by the Larmor equation. In NMR
imaging the selective pulses are applied in the
presence of localizing magnetic field gradients
(discussed herein below). Nonselective pulses
generally affect all of the nuclear spins situated
within the field of the RF pulse transmitter coil
and are typically applied in the absence of
localizing magnetic field gradients.

Upon cessation of the RF excitation, magneti-
ization $M$ due to the excited nuclear spins begin to
return to equilibrium under the influence of the $B_0$
field. As it does so, the magnetic flux intercepts
the conductors of a RF pickup coil and induces
therein a voltage, termed the NMR signal. This
return to equilibrium has associated therewith
two exponential time constants associated with
longitudinal and transverse magnetizations. The
time constants characterize the rate of return to
equilibrium of these magnetization components
following the application of perturbing RF pulses.
The first time constant is referred to as the spin-lattice relaxation time (T₁), and is the constant for the longitudinal magnetization to return to its equilibrium value. Spin-spin relaxation time (T₂) is the constant for the transverse magnetization to return to its equilibrium value in a perfectly homogeneous field B₀. In fields having inhomogeneities, the time constant for transverse magnetization is governed by a constant denoted T₂*, with T₂* being less than T₂. The values of spin-lattice and spin-spin relaxation times for protons vary widely with tissue type. For biological tissue T₁ and T₂ values may range from 30 msec. to 3 sec., and 5 msec. to 3 sec., respectively.

There remains to be considered the use of magnetic field gradients to encode spatial information (used to reconstruct images, for example) into NMR signals. Typically, three such gradients are necessary:

\[ G_x(t) = \frac{\partial B_x}{\partial x} \]
\[ G_y(t) = \frac{\partial B_y}{\partial y} \]
\[ G_z(t) = \frac{\partial B_z}{\partial z} \]

The Gₓ, Gᵧ, and Gₗ gradients are constant throughout the imaging slice, but their magnitudes are typically time dependent. The magnetic fields associated with the gradients are denoted, respectively, bₓ, bᵧ, and bₗ, wherein

\[ b_x = G_x(t)x \]
\[ b_y = G_y(t)y \]
\[ b_z = G_z(t)z \]

within the volume.

In general, T₁ relaxation time is measured by means of either a progressive saturation or an inversion recovery technique, while T₂ relaxation time is typically measured by multiple spin-echo technique. These techniques, which are well known to those skilled in the art, will be described hereinbelow in the context of a two-dimension Fourier transform (2DFT) NMR Imaging Technique (commonly referred to as spin warp).

The progressive saturation technique can be employed using four spin-warp pulse sequence which can be designated 1—4, 5—8, 9—12, and 13—16, along the horizontal axis. A complete pulse sequence (scan) would typically consist of three amplitudes (128, 256, or 512) which are substantially identical to one another, with the exception that a different amplitude of the phase-encoding pulse gradient Gₓ is employed in each (assuming no averaging).

One example comprising intervals 1—4 will now be described in detail. In interval 1, a selective 90° RF excitation pulse is applied in the presence of a positive Gᵧ gradient pulse so as to preferentially excite nuclear spins in a predetermined region of a sample object having precession frequencies as predicted by the Larmor equation. A negative Gₓ pulse is applied in interval 2 to rephase nuclear spins excited in interval 1. Typically, the Gₓ pulses are selected such that the time interval of the gradient pulse waveform over interval 2 is equal to a negative one half of the time interval of the gradient pulse over interval 1. Gₓ and Gᵧ gradient pulses are also applied simultaneously with the Gₓ gradient pulse in interval 2. The function of the Gₓ gradient pulse is, as alluded to hereinabove, to encode phase information into the excited nuclear spins. The purpose of the Gᵧ gradient pulse is to dephase the excited nuclear spins by a predetermined amount to delay the occurrence of the NMR spin-echo signal in interval 4. The spin echo is produced by the application of a 180° RF pulse in interval 3. The spin echo is sampled in interval 4 in the presence of a linear Gₓ readout gradient. The NMR information encoded in the NMR signal by the phase encoding and the readout gradient is recovered in a well-known manner using two-dimensional Fourier transform techniques.

The excitation/sampling process described hereinabove is repeated in each of the pulse sequences until the Gₓ gradient is sequenced through its range of amplitudes (128, 256, etc.). The repetition time TR, is the period of time between the beginning of one pulse sequence view and the beginning of a succeeding (essentially identical) pulse sequence of the next. TR is measured between the mean application of 90° RF pulses in succeeding steps. Typically, TR is not varied in the course of a single scan. However, TR can be varied from one scan to the next. If TR is selected to be equal to or greater than approximately five times the T₁ constant of a sample, then all of the longitudinal magnetization will have returned to equilibrium and the image resulting from such a scan would have little or no dependence on T₁. Shortening TR to be less than five times T₁ increases the T₁ contribution in the image.

The pulse sequence may be modified, for example, to include magnetic field gradient pulses immediately preceding and following the 180° RF pulses (such as the ones in intervals 3, 7, 11..., etc.) to reduce the effects of spurious NMR signals produced when regions in the sample object experience less than 180° RF excitation. These gradient pulses (termed pre-crusher and crusher) would be applied in the direction in which most of the sample is disposed. In the case of a whole-body system utilizing a solenoidal magnet, the Gₓ gradient would be pulsed. Another way in which the pulse sequence can be modified is to use selective 180° RF pulses so as to lessen the sampling bandwidth requirements thereby to reduce aliasing artifacts. Such selective 180° RF pulses would be applied in the presence of a Gₓ magnetic field gradient pulse.

In a typical pulse sequence, the duration to the Gₓ gradient in interval 1 is approximately 4 msec., while the duration of the 90° RF pulse (typically modulated by a Sin x/x function) is about 3.2 msec. from beginning to end. Similarly, the Gₓ, Gᵧ, and Gₗ gradients in interval 2 are applied for approximately 4 msec. The readout Gₓ gradient in interval 4 is selected to be approximately 8 msec. long. Magnetic field gradient pulses preceding
and following the 180° RF pulses, if used, are each 2—4 msec. The 180° RF pulse is selected to have approximately twice the amplitude of the 90° RF pulse and is also 3.2 msec. long. It will be appreciated, therefore, that a typical TR time cannot be shorter than the sum of the various times indicated, plus various power supply and amplifier recovery times. This implies that TR is typically about 30—33 msec.

The inversion recovery technique for introduction a T₁ dependence in an NMR image employs a 180° RF pulse prior to the 90° RF excitation pulse. Thus, a 180° RF pulse applied in interval 1 is followed in interval 2 by the 90° RF pulse. The time between the mean application of the 180° and the 90° pulses is referred to as inversion time (denoted TI). Variable inversion times can be selected, although a constant value is used in any given scan. As is known, the effect of the 180° RF pulse is to invert the longitudinal magnetization. The extent to which the longitudinal magnetization recovers is detected by the 90° pulse. As may be anticipated, the degree of recovery is dependent on the inversion time and the T₁ constant of the sample object. It will be apparent, therefore, that varying TI will introduce a different degree of T₁ dependence into the NMR signal and, hence, the image. As before, a magnetic field gradient pulse can be applied (e.g., a 4 msec. long G2 gradient pulse) during TI to reduce the effects of any spurious NMR signals following the 180° RF pulse.

In an inversion recovery pulse sequence, the time (denoted TE) of occurrence of the spin-echo signal is reduced to a minimum and a TR of the order of 1—2 seconds is selected. The duration of the 180° RF pulse (which need not be modulated by a Sin x function) in interval 1 may be between .250 and .700 msec.

A multiple spin-echo sequence may be used to acquire T₂ image information. This pulse sequence is substantially identical to the previously described pulse sequence with the exception that multiple spin-echo signals are produced in intervals 6 and 8 by the application of 180° RF pulses in intervals 5 and 7. The spin-echo signals occur at echo times designated TE1, TE2 and TE3. As before, succeeding spin-echo signals are sampled in the presence of contemporaneously applied linear readout G2 gradient pulses. As suggested by the "T₂ Decay" the spin echo amplitudes decay with a T₂ time constant. Of course, it will be recognized that fewer or more spin-echo signals can be produced by decreasing and increasing the number of 180° RF pulses. The maximum useful number of spin-echo signals is limited by the T₂ relaxation time of the sample object.

The pulse sequences described hereinbefore are utilized for imaging sample objects such as biological tissue having what will be referred to as "normal" T₁ and T₂ relaxation constants. These pulse sequences, particularly the timing parameters, must be modified when imaging sample objects which are under the influence of particle systems which tend to shorten normal T₁ and T₂ values. Thus, when considering, e.g., spin-echo imaging the pulse sequence, the echo time TE should be less than T₂ to achieve reasonable signal intensity. To image short relaxation time complexes, it is necessary to correspondingly shorten TE as T₁, T₂ are decreased. The minimum echo time is established by the cumulative time requirements of the pulse sequence as determined by the gradient pulses, 180° RF pulse, pre-crusher and crusher pulses and the readout signal gradient requirement. These minimum echo times total, in a conventional pulse sequence, to about 16 msec. for a 0.5T and 33 msec. for a 1.5T magnet system.

Several approaches may be taken to compress the echo time for short relaxation characteristic particle system imaging. Consider operating at the same RF field strength and using a 180° RF pulse (short or long) to match these for conventional body imaging, e.g., 1.5T. Consider further that the TE of the complex may be in the range of 1/3 to 1/10 that for biological tissue or e.g., 3.3—11 msec. and that the pulse sequence is uniformly compressed. The gradient and RF pulses must then deliver the same energy in 1/3 to 1/10 the time and, therefore, require peak pulse amplitudes between 3 and 10x the requirement for a conventional TE of about 33 msec.

Alternatively, the main field B₀ can be reduced a factor of 3—10x to a value of 0.15—0.5T for the short echo time image allowing the RF and gradient pulse power amplitudes in the time compressed pulse sequence to remain at the same peak value as employed for biological tissue imaging at e.g., TE=33 msec. This approach is less costly as it is less demanding of pulse power amplifiers. The trade-off, however, is the delay introduced in switching the B₀ field.

To image the long relaxation characteristic materials, consider TE values of 3—10x the conventional TE minimum of 33 msec. Operating at a field strength of 1.5T requires a reduction in RF and gradient pulse amplitudes by a factor of 3—10x and a uniformly stretched pulse sequence.

In utilizing the complexes of the present invention the presence and locations of lesions may be identified by injecting the complex into a mammalian host, waiting a suitable period for the complex to concentrate in the lesion, and then imaging the host twice. Once using a conventional pulse sequence to establish a reference image, and then a second time using a tailored pulse sequence (short or long) to match the short or long relaxation time characteristic of the particular complex system employed.

A water-soluble anionic coordination-complex of the trivalent, paramagnetic-ion gadolinium and bis - dihydroxyphosphonyl methyl-phosphinic acid (BDP) is prepared using the above described method and has the formula:
The above compound (Gd BDP) is employed as a contrast agent for NMR imaging. The metal-chelating ligand bis-dihydroxyphosphonylmethyl-phosphinic acid was found not to be readily hydrolyzable by the usual, phosphate hydrolyzing enzymes abundant in living tissues. Additionally, at a magnetic field of 0.47 Tesla (T), the contrast enhancing capability measured by 1/T1 of the bis-dihydroxyphosphonylmethyl-phosphinic acid-chelated gadolinium in NMR analysis using the inversion recovery method is higher than that of uncomplexed gadolinium (Gd³⁺). These results are unexpected in that ordinarily when a paramagnetic metal ion is chelated by an organic ligand, it is expected that the relaxation enhancement effect on the surrounding water molecules in the tissue of a mammalian host would be significantly reduced. Since this effect is the source for the contrast enhancement in NMR imaging, such a reduction results in a decreased contrast capability of the complexed metal ion relative to the uncomplexed one. As noted previously, complexing of the paramagnetic metal ions is effected in order to reduce the toxicity of such metal ions in mammalian hosts. The trade-off in forming the complex is to obtain contrast agents that are less toxic but still have a measurable effect on NMR imaging. It was unexpected to find that the gadolinium complex of this dihydroxyphosphonylmethylphosphinic acid has an effect which is not less but rather more pronounced (i.e. shorter relaxation times) than uncomplexed gadolinium in NMR imaging.

At 9.4T, Gd BDP relaxation times were found to be higher than Gd³⁺. Similar results were noted for Gd EDTA (ethylene diamine tetraacetic acid) complexes at both 0.47T and 9.4T.

In addition to its application as a contrast agent for NMR imaging, the complexes described and disclosed herein can be used in any scientific or technological context in which magnetic differentiation between compartments is needed. As an illustration, but not by way of limitation, these complexes are useful in NMR spectroscopic studies of membrane transport of various metabolites.

**Claims**

1. Use of the complexes of
   a) a metal ion selected from metal ions having atomic number 21 to 29 inclusive, 42 to 44 inclusive and the lanthanides having atomic numbers 57 to 70 inclusive, and
   b) an organophosphorous metal ion chelating ligand comprising a phosphonylorgano phosphinate to form complexes of the formula:

\[
[Gd(O-P-CH_2-P-CH_2-P-O)_{n}]^{(5a-5)^-}
\]
8. The use of any of claims 1 to 7 wherein the complex is used in an amount sufficient to alter the spin-lattice spin-spin or spin-echo relaxation times of an NMR signal.

Patentansprüche

1. Verwendung von Komplexen aus
   a) einem Metallion, ausgewählt aus Metallionen mit den Atomzahlen 21 bis 29 einschließlich, 42 bis 44 einschließlich und den Lanthaniden mit den Atomzahlen 57 bis 70 einschließlich, und
   b) einem Organophosphormetallation-Gelatinierungsmittel - Liganden aus einem Phosphorylorgano-
      phosphinat unter Ausbildung von Komplexen der Formel:

\[
\begin{align*}
\{&O \quad P \quad R_1 \quad P \quad O^- \} \\
&[a(n+3)^-] + v
\end{align*}
\]

worin bedeuten
n=2 bis etwa 100;
\( n_1 \)= die Zahl der negativen Ladungen in den

\[
O
\]

n = \{P - R\} - Gruppen;

\[
R_1
\]

a=1, 2 oder 3;
M= eines der vorgenannten Metallionen;
v= die Wertigkeit von M;
\( R_1 = O^- \) oder ein Organophosphinat umfassend

\[
\begin{align*}
O \\
- R \quad P \quad O^- \\
O^- 
\end{align*}
\]

worin R bedeutet: Alkyl, verzweigt oder geradkettig mit 1 bis 3 Kohlenstoffatomen;
einen zyklischen Kohlenwasserstoff mit 3 bis etwa 10 Kohlenstoffatomen, wobei der zyklische Kohlenwasserstoff gesättigt oder ungesättigt ist und im Ring kondensierte Kohlenwasserstoffe einschließt;
heterozyklische Kohlenwasserstoffe mit 1 bis 2 heterozyklischen Stickstoff-, Phosphor-, Schwefel- oder Sauerstoffatomen in dem heterozyklischen Ring, wobei der heterozyklische Ring gesättigt oder ungesättigt ist;
in einem Verfahren zur NMR-Analyse.

2. Verwendung gemäß Anspruch 1, bei welcher das Phosphinat bis Dihydroxyphosphonyl - alkyl - phosphin - oxid ist, worin die Alkylgruppe:

\[
\begin{align*}
&[Gd(O-O \quad CH_2 \quad P \quad CH_2 \quad P \quad O^-)_{n}] \\
&[a(n+3)^-] + v
\end{align*}
\]

8. Verwendung gemäß Ansprüchen 1 bis 7, in welcher der Komplex in einer ausreichenden Menge angewendet wird, um die Spin - Gitter-, Spin-Spin- oder Spin - Echo - Relaxationszeit eines NMR-Signals zu ändern.

Revendications

1. Utilisation des complexes de:
   a) un ion métallique choisi parmi les ions métalliques possédant un numéro atomique de
      21 à 29 inclus, de 42 à 44 inclus, et des lanthanides possédant des numéros atomiques de 57 à 70
      inclus; et
   b) un ligand organophosphoré, chélatant l’ion métallique, consistant en un phosphorylorgano
      phosphinate, pour former des complexes de formule:

\[
\begin{align*}
\{&O \quad O \quad P \quad R_1 \quad P \quad O^- \} \\
&[a(n+3)^-] + v
\end{align*}
\]

ou:
\( n=2 \) à environ 100;
\( n_1 \)= le nombre de charges négatives dans les n
groupes

\[
O
\]

\[
- \{P - R\} \\
R_1
\]

a=1, 2 ou 3;
M= un ion métallique décrit ci-dessus;
v= la valence de M;
R<sub>i</sub>=O<sup>-</sup> ou un organophosphénate consistant en:
\[ \text{O} \]
\[ \text{=R-P-O} \]
\[ \text{O} \]

R=alkyle, ou bien à chaîne ramifiée, ou bien à chaîne droite, contenant de 1 à 3 atomes de carbone;
hydrocarbure cyclique ayant de 3 à environ 10 atomes de carbone, ledit hydrocarbure cyclique étant saturé ou insaturé et incluant les hydrocarbures cycliques à noyaux condensés; hydrocarbures hétérocycliques ayant de 1 à 2 atomes d'azote, de phosphore, de soufre ou d'oxygène hétérocycliques dans ledit noyau hétérocyclique, ledit hydrocarbure hétérocyclique étant saturé ou insaturé,
dans un procédé d'analyse par RMN.

2. Utilisation selon la revendication 1, dans laquelle le phosphinate est l'oxyde de bis-dihydroxyphosphoryl - alkyl - phosphine, où la fraction alkyle contient un alkyle à chaîne droite ou à chaîne ramifiée, ayant de 1 à 3 atomes de carbone.

3. Utilisation selon la revendication 1, dans laquelle ledit ligand phosphinate est l'oxyde de tris-dihydroxyphosphoryl - alkyl - phosphine, où la fraction alkyle contient des alkyles à chaîne droite ou à chaîne ramifiée, ayant de 1 à 3 atomes de carbone.

4. Utilisation selon la revendication 2, dans laquelle ledit ligand phosphinate est l'acide bis-dihydroxyphosphoryl - méthyl phosphonique.

5. Utilisation selon la revendication 3, dans laquelle ledit composé du phosphore est l'oxyde tris-dihydroxyphosphoryl méthyl phosphonique.

6. Utilisation selon la revendication 1, dans laquelle R représente méthyle.

7. Utilisation selon l'une des revendications 1 à 6, dans laquelle le complexe présente la formule:

\[ \text{[Gd(O-P-CH}_{2}\text{P-CH}_{2}\text{P-O]}_{n}} \]
\[ \text{O} \text{O} \text{O} \]
\[ \text{O} \text{O} \text{O} \]

8. Utilisation selon l'une des revendications 1 à 7, dans laquelle le complexe est utilisé en une quantité suffisante pour modifier les temps de relaxation spin-réseau, spin-spin ou spin-écho d'un signal de RMN.