



(11) **EP 2 799 090 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

- (15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 25-29, 31, 41-42,
58, 60-62, 87
Claims DE 1, 8
Claims EN 1, 8
- (51) Int Cl.:
A61K 49/10^(2006.01) A61K 51/04^(2006.01)
- (48) Corrigendum issued on:
21.12.2016 Bulletin 2016/51
- (45) Date of publication and mention of the grant of the patent:
29.06.2016 Bulletin 2016/26
- (21) Application number: **14165008.5**
- (22) Date of filing: **18.02.2009**

(54) **Process for preparing a pharmaceutical formulation of contrast agents**

Verfahren zur Herstellung einer pharmazeutischen Formulierung aus Kontrastmitteln

Procédé de préparation d'une formulation pharmaceutique d'agents de contraste

- (84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK TR
- (30) Priority: **19.02.2008 FR 0851055**
17.04.2008 EP 08154745
12.06.2008 US 155997
- (43) Date of publication of application:
05.11.2014 Bulletin 2014/45
- (62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
13154448.8 / 2 591 807
09712403.6 / 2 242 515
- (73) Proprietor: **GUERBET**
93420 Villepinte (FR)
- (72) Inventors:
• Meyer, Dominique
17000 La Rochelle (FR)
- **Corot, Claire**
69006 Lyon (FR)
- **Port, Marc**
95170 Deuil La Barre (FR)
- **Barbotin, Vincent**
95160 Montmorency (FR)
- **Bonnemain, Bruno**
77270 Villeparisis (FR)
- (74) Representative: **Regimbeau**
20, rue de Chazelles
75847 Paris Cedex 17 (FR)
- (56) References cited:
EP-A1- 0 481 526 WO-A-89/00052
WO-A-2007/121453 US-A- 4 647 447
US-A- 5 876 695 US-A1- 2004 170 566

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 2 799 090 B9

- **COROT C ET AL:** "Structure-activity relationship of macrocyclic and linear gadolinium chelates: Investigation of transmetallation effect on the zinc-dependent metallopeptidase angiotensin-converting enzyme", **JOURNAL OF MAGNETIC RESONANCE IMAGING** 199805 US, vol. 8, no. 3, May 1998 (1998-05), pages 695-702, XP008097347, ISSN: 1053-1807
- **KIMURA J ET AL:** "Human comparative study of zinc and copper excretion via urine after administration of magnetic resonance imaging contrast agents", **RADIATION MEDICINE - MEDICAL IMAGING AND RADIATION ONCOLOGY** 200508 JP, vol. 23, no. 5, August 2005 (2005-08), pages 322-326, XP002499275, ISSN: 0288-2043
- **ZHU X ET AL:** "Formation kinetics and stability studies on the lanthanide complexes of 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetraacetic acid by capillary electrophoresis", **ELECTROPHORESIS** 2002 DE, vol. 23, no. 9, 2002, pages 1348-1356, XP008097346, ISSN: 0173-0835
- **ANONYME:** "NEPHROGENIC SYSTEMIC FIBROSIS: AN UNCOMMON AND DEBILITATING DISEASE POSSIBLY ASSOCIATED WITH GADOLINIUM CHELATES", **GUERBET INFORMATION FOR HEALTHCARE PROFESSIONALS AND INVESTORS** , 21 December 2007 (2007-12-21), pages 1-7, XP002499276, Villepinte, France Retrieved from the Internet:
URL:http://www.guerbet.com/fileadmin/user_upload/news/Updated_information_about_NSF_21_dec_2007.pdf [retrieved on 2008-10-13]

Description

[0001] The invention discloses pharmaceutical formulations of contrast agents, in particular of complexes of chelates with paramagnetic metal ions, especially for magnetic resonance imaging, and to industrially efficient processes for obtaining these formulations.

[0002] Many contrast agents based on complexes of chelates with lanthanides (paramagnetic metal), in particular with gadolinium, are known, and are described, for example, in document US 4 647 447. Several products are marketed, especially based on macrocyclic chelates such as DOTA gadoterate (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid) and gadoteridol HPDO3A, and linear chelates such as DTPA (diethylenetriaminepentaacetic acid) and DTPA-BMA (gadodiamide).

[0003] In the body, the complexes of chelates with lanthanide are in a situation of chemical equilibrium, which may lead to a risk of undesired release of the lanthanide, and more especially of gadolinium. A person skilled in the art is thus led to seek technical solutions that limit this risk in order to solve the complex problem of tolerance in the patient as safely as possible. This problem is all the more difficult since the administration of contrast agents is often repeated during diagnostic examinations and/or for the guiding and monitoring of the efficacy of a therapeutic treatment.

[0004] Several approaches for improving the tolerance of complexes of chelates with gadolinium are described in the prior art.

More than twenty years ago (US 5 876 695), those skilled in the art were working on formulations consisting of the addition to a lanthanide-complexing chelate of an amount of chelate in excess, i.e. chelate non-complexed by the lanthanide. This excess chelate is intended to compensate for an undesired release of lanthanide, the excess chelate then complexing with the released lanthanide (Gd^{3+} metal ion).

[0005] In US 5 876 695 the chelates (ligands L) added in excess for macrocyclic chelates are described under the form of an excipient having the formula $X[X',L]$, where X and X' are metal ions (especially calcium, sodium, zinc or magnesium) and L is the chelate in excess. These excipients are designed to scavenge free lanthanide.

[0006] For instance for the chelate DOTA, an excipient is $Na_2[Ca-DOTA]$: the DOTA chelate in excess is complexed by the calcium ion Ca^{2+} in the cage formed by the chelate, with a resulting charge 2+ to be neutralised by two Na^+ ions.

[0007] A few years later, an improvement of these excipients $X[X',L]$ was presented in the patent EP 454 078 (US 7 385 041) with improved $X[X',L]$ where both X and X' are calcium or zinc, these excipients being able even at low dosage (0,1% mol/mol) to scavenge both free lanthanide and free organic ligand chelate. This document covers these excipients, in particular for example the calcium salts of calcium chelated complex, notably $Ca[Ca-HPDO3A]_2$ instead of $Na[Ca-HPDO3A]$, and explains (in detail notably column 1 lines 21-40) that a free macrocyclic ligand L instead of such excipient $X[X',L]$ should not be used for safety reasons due to the toxicity of free chelate L.

[0008] In particular, table 1 of US 7 385 041 illustrates with LD50 values that free macrocyclic chelates (HP-DO3A, DO3A, DOTA) are about at least 10 times more toxic than these macrocycles under the form $X[X',L]$. In particular for DOTA, the LD50 is at least about 40 times better for $Na_2[Ca-DOTA]$ than for free DOTA.

	Chelate	Letal dose (LD 50) mMol/Kg
Free macrocyclic chelate L (not used as excess ligand)	HP-DO3A	0.11
	DO3A	0.12
	DOTA	0.18
Excipient $X[X',L]$	$Ca[Ca-HPDO3A]_2$	1.3
	$Ca[Ca-DO3A]_2$	1.6
	$Na_2 [Ca-DOTA]$	> 7
Chelate-Gd	DOTA-Gd	14
	HP-DO3A-Gd	12

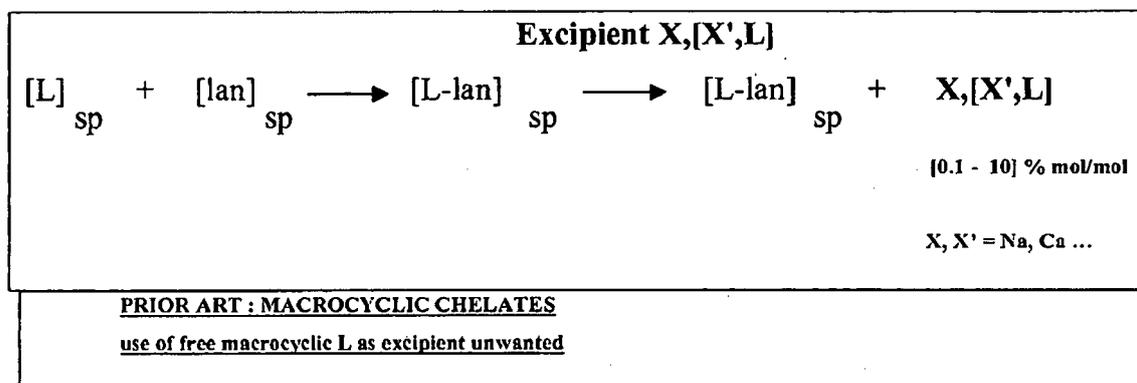
[0009] The formulation of the commercialised product gadoteridol (Prohance Bracco) includes 0.1% $Ca[Ca-HPDO3A]_2$, and gadobutrol product (Gadovist Bayer Schering) includes $Na[Ca-BT DO3A]$ excipient.

[0010] Corot et al (Journal of Magnetic Resonance Imaging, vol 8, n°3, May 1998, pages 695-702) describes the use in the product gadoteridol (Prohance®) of a chelate excess under the form of an excipient of formula $Ca[Ca-HPDO3A]_2$ in which the cation Ca^{2+} of the complex $Ca-HPDO_3A$ is complexed by the chelate, and the other cation Ca^{2+} is used to obtain a salt complex $[Ca-HPDO_3A]_2$. This formulation thus contains a chelate salt.

[0011] As a conclusion, no document of the prior art describes that the formulation of a macrocyclic chelate administered to the patient contains or should contain, besides the macrocyclic chelate complexed by the lanthanide, an excess of free macrocyclic chelate (in a specific and low range) that is under the form of a free chelate L which was not complexed

with any metal ions and in particular that is not under the form of an excipient X[X',L]. On the opposite the one skilled in the art was discouraged to do so due to a risk in terms of tolerance of free macrocyclic chelate.

[0012] It is also emphasized that in the prior art for the macrocyclic chelate (contrarily to the invention as described later), the dedicated excipient X[X',L] was added after the complexation of the chelate by the lanthanide (see the numerous examples of US 5 876 695 and US 7 385 041). The complexation was realised according to the stoichiometric proportions of the chelate L (HP-DO3A for example) and lanthanide "lan" (Gd3+ for example). Following **scheme I** describes the manufacturing process of the prior art (sp means stoichiometric proportions) :



[0013] Despite all these prior-art studies, the complex problem of tolerance still exists, especially in situations at risk of more pronounced tolerance for the administration of MRI contrast products. For instance a very different approach was tested recently as illustrated in WO 2007/106 544 with grafting onto the chelates chemical groups intended to increase the affinity of the chelate for the metal.

[0014] A new problem has moreover recently appeared in the matter of tolerance, namely a pathology known as NSF (nephrologic systemic fibrosis, or fibrogenic dermopathy, with very severe effects on human skin), which may be at least partly correlated to the existence of free gadolinium, i.e. non-complexed gadolinium, in the body. This disease has led to health authorities being alerted as regards certain categories of patients with respect to marketed gadolinium-based contrast agents. Briefly, NSF could be associated to the transmetallation of some lanthanide from the complex [lanthanide-chelate] by endogenous ions such as zinc and resulting in unwanted release of free lanthanide.

[0015] In summary, this problem of tolerance of complexes of chelates with lanthanides remains complex and important, leading to the research of even more safe products, and to the necessity of a perfectly controlled rate of the different entities in the pharmaceutical solutions.

[0016] The Applicant has worked on the specific case of macrocyclic chelates, and has demonstrated, contrary to what was expected, the very satisfying tolerance obtained when using an amount of excess free macrocyclic chelate at a particular low dose range, and not under the form of an excipient X[X',L] of the prior art.

[0017] The Applicant has shown that with macrocyclic chelates, and in particular DOTA, results are very advantageous, using a very low excess of free chelate L, so that the pharmaceutical composition administered to the patient contains more specifically between 0.02% and 0.4% and in particular between 0.025% and 0.25% of the free macrocyclic chelate L.

[0018] For the purposes of the present invention hereafter, the term "free macrocyclic chelate" means any macrocyclic chelate L not complexed with lanthanide or with other metal ions, and in particular not under the form of an excipient X[X',L] in which X and X' are as described above.

[0019] Briefly the formulation selected by the Applicant with free macrocycle has the strong advantage, notably in view of the NSF, of increasing highly the scavenging capacity of potential free gadolinium, as compared to the prior excipients X[X',L], as explained further in detail in the application.

[0020] Consequently, in view of this low amount of free excess, a new problem arises, which is unknown in the prior art, namely the need for extremely precise and delicate industrial-scale control of the concentrations of free macrocyclic chelate and thus of the manufacture of the product to arrive at this range of target values of amount of free chelate, these values needing to be stable, including after storage for several months or years.

[0021] Specifically, taking into account the production volumes, which are of the order of several tens of tons of active principle, the Applicant had to develop a new and particularly optimised preparation process that makes it possible to ensure the reliability and reproducibility of the composition of commercialized batches.

[0022] In particular, the Applicant found that the mixing of stoichiometric amounts on the basis of the theoretical calculation does not sufficiently satisfactorily give at the industrial scale the respective amounts of complex of chelate with the lanthanide and of free chelate in low concentration in the pharmaceutical formulation. The reason for this is that it is then necessary to perform several analysis steps, which takes several hours, and significantly increases the industrial

cost price of the product. In contrast, the Applicant's process makes it possible especially to prepare beforehand and to optimize the analytical device, which is important as regards its impact on the quality of the final product.

[0023] More specifically, by respecting the stoichiometric proportions and by adding an excess of DOTA intended not to be complexed with the lanthanide, it is not possible at the industrial scale to achieve sufficiently reproducibly in the final pharmaceutical solution an excess of free DOTA in the target range, especially given:

- 1) the uncertainty of weighing at the industrial scale, which does not make it possible to correctly ensure the ratio (of the order of 1000) between the chelate and the excess chelate, given the small amount of excess chelate;
- 2) the variability of the hygroscopic characteristics of the chelate (associated with its acid functions).

[0024] It is pointed out, specifically, that to prepare an industrial amount, typically, for example 200 litres of a 0.5 M solution of gadolinium chelate (for example DOTA-Gd), the amount of DOTA to be added in excess after complexation of the DOTA with the lanthanide, to obtain an excess of free DOTA of 0.1 mol/mol%, would be about 40 g of DOTA in 200 litres of the DOTA solution (40 g in addition to the 40 kg of DOTA initially placed in solution), which does not allow sufficiently reliable reproducibility at the industrial level.

[0025] This problem has been solved by the Applicant by means of using at least one step of measuring in the liquid pharmaceutical formulation concentrations of free macrocyclic chelate ($C_{ch\ I}$) and/or of free lanthanide ($C_{lan\ I}$) and at least one step of adjusting the $C_{ch\ I}$ and/or the $C_{lan\ I}$ so as to obtain the desired concentration of $C_{ch\ I}$ and $C_{lan\ I} = 0$, advantageously by modifying the amounts of macrocyclic chelate or of lanthanide in the pharmaceutical composition.

$C_{ch\ I}$ abbreviation refers to the concentration of free chelate. $C_{lan\ I}$ abbreviation refers to the concentration of free lanthanide.

[0026] Throughout the application, the equality $C_{lan\ I} = 0$ is used to define that $C_{lan\ I}$ in the formulation injected into the patient is zero or substantially zero (typically less than 10^{-10} M and advantageously less than 10^{-12} M or 10^{-14} M), the possible presence in solution of an extremely small amount of lanthanide not being able to be totally excluded. The reason for this is that concentrations less than 10^{-10} M cannot be measured sufficiently reliably by the current analytical methods.

[0027] Thus, the present invention relates to a process for preparing a liquid pharmaceutical formulation of complex of macrocyclic chelate with lanthanide, the said process comprising at least one step of measuring in the liquid pharmaceutical formulation concentrations of free macrocyclic chelate ($C_{ch\ I}$) and/or of free lanthanide ($C_{lan\ I}$) and at least one step of adjusting the $C_{ch\ I}$ and/or the $C_{lan\ I}$, so as to obtain (sufficiently stably in the final pharmaceutical solution, i.e. the pharmaceutical formulation intended to be administered to the patient) a mol/mol amount of free macrocyclic chelate of between 0.002% and 0.4%, advantageously between 0.02% and 0.3% and very advantageously between 0.025% and 0.25%.

[0028] The present invention thus relates to a process for preparing a liquid pharmaceutical formulation containing a complex of macrocyclic chelate with a lanthanide and a mol/mol amount of free macrocyclic chelate of between 0.002% and 0.4%, advantageously between 0.02% and 0.3% and very advantageously between 0.025% and 0.25%, the macrocyclic chelate being DOTA and the lanthanide being gadolinium, the said process comprising the following successive steps:

b) preparation of a liquid pharmaceutical composition containing, firstly, the complex of macrocyclic chelate, with a lanthanide, and, secondly, free macrocyclic chelate, advantageously that is not under the form of an excipient $X[X',L]$ in which L is the macrocyclic chelate and X and X' are a metal ion, in particular chosen independently from calcium, sodium, zinc and magnesium, and/or free lanthanide;

said step b) consisting in the preparation of a solid complex [chelate-lanthanide] and in the dissolution of the said complex in water;

c) measurement in the pharmaceutical formulation obtained in step b) of the concentration of free macrocyclic chelate $C_{ch\ I}$ and/or of free lanthanide $C_{lan\ I}$;

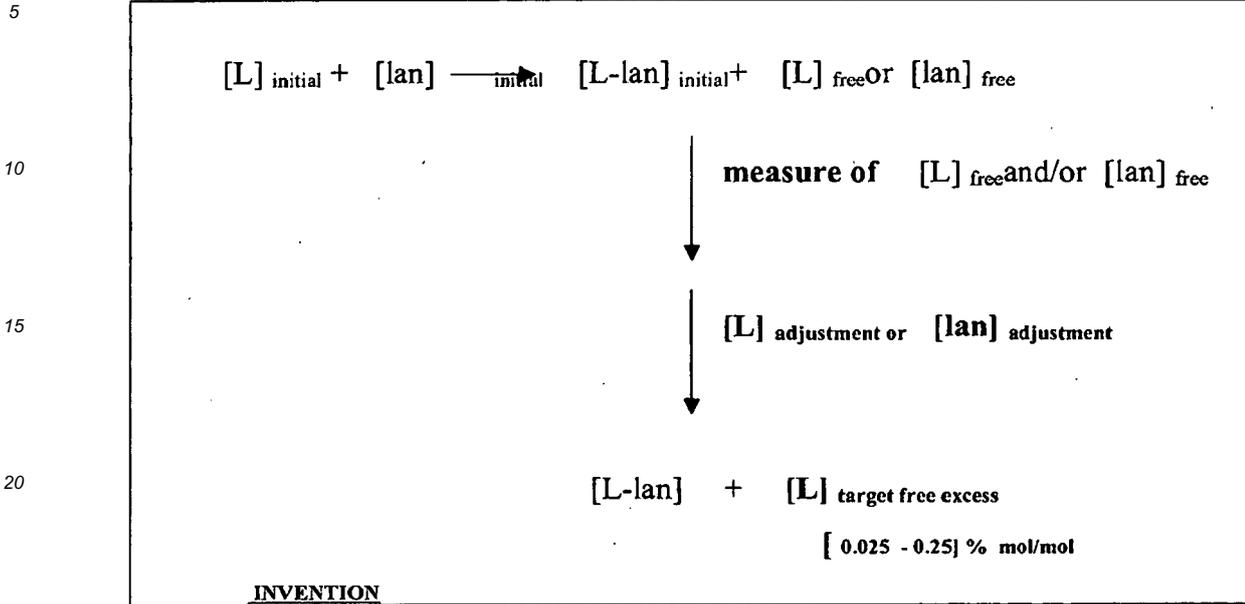
d) adjustment of $C_{ch\ I}$ and/or of $C_{lan\ I}$ so as to obtain $C_{ch\ I} = C_{t\ ch\ I}$ and $C_{lan\ I} = 0$, wherein $C_{t\ ch\ I}$ is the target concentration of the free macrocyclic chelate in the final liquid pharmaceutical formulation,

said step d) of adjustment of $C_{ch\ I}$ and/or of $C_{lan\ I}$ being carried out by adding to the formulation obtained in step b):

- if $C_{lan\ I} > 0$ and/or $C_{ch\ I} < C_{t\ ch\ I}$, free macrocyclic chelate,
- if $C_{lan\ I} = 0$ and $C_{ch\ I} > C_{t\ ch\ I}$, free lanthanide.

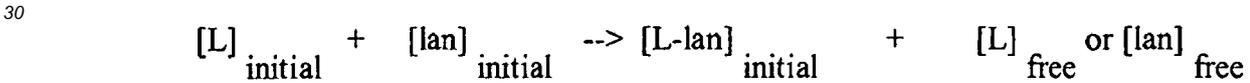
[0029] Advantageously, the process according to the present invention comprises a prior step a) of determination of the theoretical target concentration of free macrocyclic chelate $C_{t\ ch\ I}$ in the final liquid pharmaceutical formulation.

[0030] The reaction is represented as follows as **Scheme II** (with "L" the ligand chelate, and "lan" the lanthanide gadolinium Gd³⁺ for example):



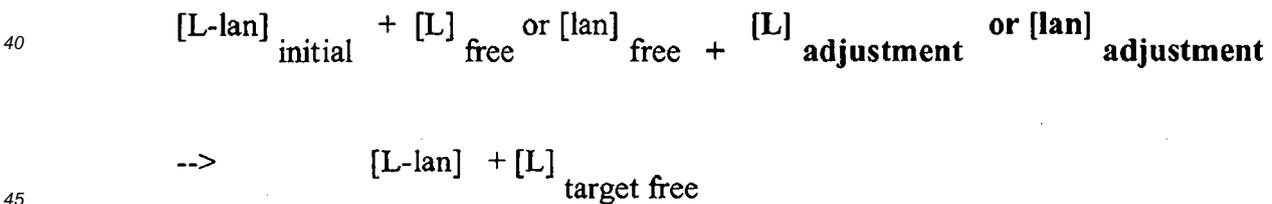
25 [0031] The reaction is in two steps :

Step 1 :



35 with the concentration C_{chl} of $[L]_{\text{free}}$ and the concentration C_{lan} of $[\text{lan}]_{\text{free}}$ being measured

Step 2 :



with the concentration $C_{\text{chl}} = C_{\text{tchl}}$ of $[L]_{\text{target free}}$ and $C_{\text{lan}} = 0$ of $[\text{lan}]_{\text{free}}$

50 [0032] For the purposes of the present invention, the term "amount of free macrocyclic chelate" means the proportion of free macrocyclic chelate relative to the amount of complexed macrocyclic chelate (gadoteric acid in the case of DOTA-Gd) present in the formulation in mol/mol. In the rest of the description, it will be referred to without preference as the "amount of free macrocyclic chelate" or the "excess free macrocyclic chelate". And as mentioned before, macrocyclic chelate L is not under the form of an excipient X[X',L] and is not complexed with any metal ion (namely X and X').

55 [0033] For the purposes of the present invention, the term "free lanthanide" means any lanthanide not complexed with a macrocyclic chelate.

It is recalled herein that in document US 5 876 695 for the linear DTPA chelate, in Example 2, the amounts of excess chelate are defined from the start on the calculated basis of the stoichiometry, and without a step of prior adjustment or measurement of the concentrations. More specifically, in the said example, 0.5 mol of DTPA and 0.25 mol of gadolinium

oxide (Gd_2O_3) are mixed in accordance with the stoichiometric proportions, and a 0.1 mol/mol% (0.5 mmol) excess of ligand is added; for macrocyclic chelates this does not ensure the target amount of ligand desired by the Applicant in the case of a large-scale industrial manufacture.

[0034] It is precised that the use of an excess of free linear ligand DTPA is destined to scavenge free lanthanide that would otherwise be liberated during the conservation time of the formulation.

[0035] The adjustment process of the invention for macrocyclics is advantageously destined to make absolutely sure (in particular due to the quantities used and to the detection limited capacities of the available analytical tools) that the level of free entities is totally controlled, and in particular that there is no free gadolinium in the manufactured pharmaceutical solution. This adjustment method is particularly advantageous for the industrial complexation issued from the mixture of the chelate and the lanthanide in solution.

[0036] It is also reminded that, as know by the one skilled in the art, the process of adjustment of the Applicant would not be applicable at the industrial scale with an excipient $X[X', L]$ in view of the thermodynamic and kinetic equilibrium of such excipient, except maybe if further very complex methods were used (the manufacturer would have to manage both the metal ions and the lanthanide kinetic and thermodynamic constants).

[0037] As device for analysis/assay of the free macrocyclic chelate, any suitable equipment is used. Advantageously, a potentiometer or capillary electrophoresis is used for the macrocyclic chelate. More specifically, in the presence of copper sulfate, the free DOTA contained in the solution obtained from the complexation step (in bulk) complexes the copper. The excess copper sulfate is assayed in preferably pH 5 buffered medium, by potentiometry, with a solution of EDTA in the presence of a copper-indicating electrode and a reference electrode.

[0038] The analysis/assay of the free lanthanide is performed by using, for example, a solution of EDTA in the presence of xylenol orange Arsenezo as turning-point indicator. Free gadolinium is assayed advantageously with a colorimetric method using 0.01 M edetate disodium titration solution in the presence of xylenol orange as indicator. Titration is carried out in pH=5 sodium acetate / acetic acid buffered solution on 20 mL of DOTAREM product until the indicator turns colour from red to yellow. 0.1 ml of 0.01 M edetate disodium solution corresponds to 0.0008 % weight/volume of free Gd (8 ppm). The method is valid from 8 to 100 ppm of free gadolinium

[0039] To the knowledge of the Applicant colorimetric methods are well known but it was neither known nor suggested to use them for the measure of gadolinium Gd^{3+} in contrast agents at the very low levels of the present invention, which is of strong interest for the adjustment process of the invention and belongs to the same inventive concept. As such the invention also relates according to another aspect to an analytical method of measuring free lanthanide at the low range of the application and consisting in a colorimetric method (also called potentiometric method).

[0040] The present invention concerns an analytical colorimetric method for measuring the level of free lanthanide in a liquid pharmaceutical formulation containing a complex of macrocyclic chelate with a lanthanide and a mol/mol amount of free macrocyclic chelate of between 0.002% and 0.4%.

[0041] In order to perform step d), several solutions are possible as a function of the $C_{lan I}$ and $C_{chl I}$ measured in step c).

[0042] In particular, the following solutions are concerned:

- if $C_{lan I} > 0$ and/or $C_{chl I} < C_{tchl I}$, the adjustment is performed by adding free macrocyclic chelate;
- if $C_{lan I} = 0$ and $C_{chl I} > C_{tchl I}$, the adjustment is performed by adding free lanthanide.

[0043] The lanthanide is advantageously added in the form of oxide (gadolinium oxide in particular), but the invention also covers other possible forms of lanthanide, especially the lanthanide salts known to those skilled in the art.

[0044] The precise experimental conditions of step b) are detailed in the examples. Advantageously, the temperature for step b) is between 60 and 100°C, and is advantageously about 80°C. Advantageously, the pharmaceutical formulation is then cooled before the adjustment step d). The duration of step b) is, for example, from 1 hour to 3 hours.

[0045] Moreover, throughout the description hereinabove and hereinbelow of the adjustment variants of step d), it is understood that the complexation step b) may be performed in several sub-steps which would be equivalent to an overall complexation step. The complexation may be performed, for example, by preparing about half the final volume of the tank, and then adding gadolinium oxide at acidic pH.

[0046] Advantageously, it is preferred, in the process according to the invention, for step c) of measurement of the concentrations to be performed in a medium in which the complexation reaction of step b) is performed:

- by using a difference between the stoichiometric proportions and the amounts of free lanthanide and of free macrocyclic chelate added in step b),
- or by modifying the pH to shift the chemical equilibria in favour or in disfavour of complexation.

[0047] For the purposes of the present invention, the expression "difference between the stoichiometric proportions and the amounts of free lanthanide and of free macrocyclic chelate added in step b)" means that the amounts of free lanthanide and of free chelate added in step b) are such that, in the light of the stoichiometry of the complexation reaction,

not all the lanthanide is complexed by the chelate (excess lanthanide and/or deficit of chelate relative to the stoichiometry) or not all the chelate is complexed with the lanthanide (excess chelate and/or deficit of lanthanide).

[0048] Advantageously, this difference is such that the macrocyclic chelate/lanthanide or lanthanide/macrocylic chelate mol/mol ratio is less than or equal to 1.4, advantageously between 1.001 and 1.3, particularly advantageously between 1.005 and 1.2, and in particular between 1.005 and 1.02. It is also pointed out that this ratio may be adapted depending on whether an excess of chelate or an excess of lanthanide is used. When an excess of lanthanide is used for the complexation, advantageously the lanthanide/macrocylic chelate mol/mol ratio is typically less than or equal to 1.2. When an excess of chelate is used for the complexation, the macrocylic chelate/lanthanide mol/mol ratio is advantageously less than or equal to 1.4.

[0049] Thus, the amounts of free macrocylic chelate and of free lanthanide added are such that not all the macrocylic chelate is complexed with the lanthanide or such that not all the lanthanide is complexed with the macrocylic chelate. Consequently, after this step b), the pharmaceutical formulation will typically comprise macrocylic chelate-lanthanide complex and:

- either free macrocylic chelate,
- or free lanthanide.

[0050] In this case, the preparation process according to the present invention is characterized in that, in step b), there is a difference between the amounts of free macrocylic chelate and of free lanthanide added and the stoichiometric proportions, this difference advantageously being such that the macrocylic chelate/lanthanide or lanthanide/macrocylic chelate mol/mol ratio is less than or equal to 1.4, advantageously between 1.001 and 1.3, particularly advantageously between 1.005 and 1.2 and in particular between 1.005 and 1.02.

[0051] According to particular embodiments, the ratio will be, for example, 1.01, 1.02, 1.03 or 1.04. This gives, for example, the concentrations presented in Table 1 below, which shows the case of an excess of initial free lanthanide, given that an excess of initial chelate may also be used.

Concentration of free macrocylic chelate (initial) added in step b) (M)	Concentration of free lanthanide (initial) (1) added in step b) (M)	Lanthanide/chelate ratio
0.480	0.520	1.083
0.487	0.513	1.053
0.492	0.508	1.032
0.497	0.504	1.014
(I) this is the amount of Gd ³⁺ , and not of Gd ₂ O ₃		

[0052] For the chelate DOTA, an amount of free chelate corresponding to a concentration of 0.497 M of chelate and an amount of free lanthanide corresponding to a concentration of 0.504 M of lanthanide, which corresponds to a lanthanide/DOTA mol/mol ratio of $x=1.014$ (with $x = 0.504/0.497$), will be added in step b).

[0053] Another way of expressing this difference relative to the stoichiometric proportions is to define it relative to the lanthanide concentration in the final solution.

[0054] In the case of this example which illustrates an excess of lanthanide, the difference is $0.6\% = 100 * [(0.5 - 0.497)/0.5]$, for a formulation at 0.5 M of gadolinium at stoichiometry. The difference is thus, for example, advantageously between 0.1 mol% and 2 mol% of the concentration at stoichiometry of the pharmaceutical formulation.

[0055] In one embodiment that is also advantageous (preferred mode) the adjustment step d) is performed without touching the total amount of lanthanide present in the formulation, i.e. without adding or removing any lanthanide. In this case, only the total amount of macrocylic chelate and/or the pH is modified.

[0056] For the purposes of the present invention, the term "total amount of lanthanide" means all the lanthanide present in free form and in complexed form.

For the purposes of the present invention, the term "total amount of macrocylic chelate" means all the macrocylic chelate present in free form and in complexed form.

[0057] Thus, in a first case (case A of the preferred mode), an excess of lanthanide relative to the macrocylic chelate is added in step b) ; and step d) consists in adding free macrocylic chelate.

[0058] In this case, the preparation process according to the present invention is characterized in that:

- in step b), the amounts of free macrocylic chelate and of free lanthanide added are such that not all the lanthanide is complexed, the lanthanide/macrocylic chelate ratio (mol/mol) advantageously being less than 1.2;

- step c) consists in solely measuring of C_{lanI} , C_{chI} typically being equal to 0 (or substantially equal to 0);
- step d) consists in adding to the formulation obtained in step b) the amount of free macrocyclic chelate necessary, firstly, to complete the complexation of the free lanthanide so as to obtain $C_{lanI} = 0$ and, secondly, to obtain an excess of free macrocyclic chelate $C_{chI} = C_{tchI}$.

5

[0059] In a second case (case B of the preferred mode), an excess of macrocyclic chelate relative to the lanthanide is added in step b). In this case, depending on the excess of chelate, step d) consists in adding or removing macrocyclic chelate.

10

[0060] Specifically, when the excess chelate added in step b) makes it possible to obtain $C_{chI} < C_{tchI}$, it is then appropriate in step d) to add further free macrocyclic chelate in order to obtain $C_{chI} = C_{tchI}$.

[0061] On the other hand, if the excess chelate added in step b) makes it possible to obtain $C_{chI} > C_{tchI}$, it is then appropriate in step d) to remove free macrocyclic chelate (to add free Gd) in order to obtain $C_{chI} = C_{tchI}$.

[0062] In this latter case, the preparation process according to the present invention is characterized in that:

- in step b), the amounts of free macrocyclic chelate and free lanthanide added are such that all the lanthanide is complexed and that $C_{chI} > C_{tchI}$, the macrocyclic chelate/lanthanide ratio (mol/mol) advantageously being less than 1,2;
- step c) consists in solely measuring C_{chI} , C_{lanI} being equal, to 0;
- step d) consists in removing the appropriate amount of free macrocyclic chelate so as to obtain $C_{chI} = C_{tchI}$.

20

[0063] It is pointed out that, in cases A) and B), as illustrated in the detailed Example 2, the adjustment step d) comprises at the end a step of adjustment of the pH and of the volume, advantageously with meglumine for DOTA.

[0064] In a third case (case C) the pH of the formulation (and optionally other functionally equivalent chemical parameters) is controlled so as to shift the reaction equilibrium in order to obtain at the end the target pharmaceutical solutions (excess amount of target ligand).

25

[0065] For example, the complexation is performed at a pH below 6 (for example between 3 and 6 and advantageously between 5 and 6) and the pH is then raised, for example, to about 12 (for example with NaOH), and the pH is then adjusted to about 7.

[0066] In variants of the process without pH adjustment, as described in the detailed Example 2 of the present patent application, in step 2, the complexation is typically performed at a pH below 6 (for example between 3 and 6), the pH being brought directly to about 7. Increasing the pH makes it possible to shift the equilibrium in the direction from an excess of macrocyclic chelate to a level substantially equal to the target excess amount. Next, by reducing the pH, a reduction at a very low rate in the amount of free macrocyclic chelate is obtained such that, over the shelf life of the product, the amount of free lanthanide/macrocylic chelate does not change unfavourably. This would result from the implied thermodynamic constants associated with the pH modifications.

30

35

[0067] It may also be pointed out that when the free lanthanide measurement will be performed (at pH 7), the concentration will be lower than if the pH change had not been made, and the adjustment is then made with the correct amount of chelate.

[0068] Furthermore, without going into the detail of the complexation mechanisms that take place at the molecular level in several phases (described especially in Chem. Eur. J., 2004, 10, 5218-5232), the Applicant points out that it was not at all obvious that the process with adjustment would make it possible to obtain this result.

40

[0069] According to the invention, step b) consists in preparing a solid complex [chelate-lanthanide] and in dissolving it (in water).

[0070] In this case, step d) is performed on a liquid formulation obtained by dissolving a solid [chelate-lanthanide] complex.

45

[0071] Step b) of the process according to the invention comprises two sub-steps:

- i) the preparation of a solid complex [chelate-lanthanide] and
- ii) the dissolution of the complex obtained in step i).

50

[0072] The adjustment in step d) may be performed as described previously in detail (addition of chelate or of lanthanide, removal of chelate or of lanthanide, adjustment by pH modification).

[0073] The preparation of the solid complex, which is advantageously crystalline [chelate-lanthanide], involves, where appropriate, at least one treatment step (filtration, concentration, crystallization, drying, spraying, etc.) for obtaining the appropriate physicochemical characteristics, especially in terms of solubility and purity.

55

[0074] As a conclusion, the manufacturing methods of the Applicant allow the optimized control of the proper range of free ligand excess, which is important for the clinicians. In vivo the scavenging capacity towards free gadolinium is presumably much higher for free ligand (DOTA) than for excipient $X[X',L]$ (sodium salt of DOTA-Ca for instance). Taking

DOTA as macrocyclic chelate, considering that the kinetic of complexation / uncomplexation of an excipient X[X',DOTA] is much less that of free DOTA, this excipient would liberate the DOTA only slowly and/or a little in physiological situation, as compared to the free DOTA. Thus free DOTA of the formulation of the applicant is, as regards to free Gd complexing, significantly more available than the DOTA of the excipient X[X',DOTA], notably in case of an accumulation of complex in a biological compartment. As a result, free DOTA excess is highly much better than excipient X[X',DOTA] for avoiding the transmetallation due to free gadolinium in vivo.

[0075] It is precised that this is different from the case of linear chelates (DTPA-BMA notably), for which the excipient X[X',L] is used because this excipient uncomplexes very quickly (or leads to quick transmetallation) and thus can scavenge free Gd. This effect of excipient X[X',L] for linear chelates has been recently demonstrated in vivo on human skin NSF patients and this excipient is added in high quantity (5 to 10% mol/mol).

[0076] In addition, in another particularly advantageous embodiment, a further technical problem was solved by the Applicant for the industrial manufacture of a pharmaceutical formulation of contrast agent based on a macrocyclic chelate-lanthanide complex, while at the same time making it possible to maximize the tolerance profile of the contrast product. More specifically, contrary to the prior-art teaching, for example US 5 082 649 (excess of free calcium of 1 to 25%) which completes US 5 876 695 (which uses large quantities of calcium chelate) in the pharmaceutical formulation, the Applicant has demonstrated that, in the case of the process according to the present invention, a very low amount of calcium would make it possible to ensure the industrial control of this process and to obtain a very well-tolerated product.

[0077] More specifically, the reliability of step c) of measuring the amounts of chelate and/or of lanthanide with common industrial analytical tools is markedly improved when the amount of calcium in the components used (in particular in the macrocyclic chelate, the lanthanide and the water used in step b)) is less than a very low target value of around 15 to 200 ppm. The amount of calcium (quantity of calcium) in the macrocyclic chelate used in step b) is advantageously less than 200 ppm and advantageously in the region of or less than 50 ppm and even preferably less than 15 ppm. For example, if the amount of calcium in the DOTA [active principle in the form of powder supplemented with water in step b) - see the detailed Example 2 - dissolution step 1] is too high (and especially greater than 200 ppm), calcium may complex the chelate and the adjustment of the amount of free chelate will not be performed sufficiently satisfactorily.

[0078] The low amount of calcium in the pharmaceutical solution makes it possible to avoid possible disadvantageous interferences regarding the assays of free macrocyclic chelate (for example by complexing the calcium with the chelate) and thus to obtain an assay of the free chelate and its adjustment in a manner that is particularly effective for manufacture at the industrial scale at the required high level of quality. Furthermore, a very low calcium concentration controlled in the final product administered to the patient (especially the meglumine salt of gadolinium DOTA) is advantageous as regards the calcaemia of the patients in so far as it makes it possible to avoid any homeostasis imbalance: the impact of the injected product (typically at a dose of less than 20 ml) on the calcaemia is at most in the region of 0.5%. The amount of calcium in the administered contrast product is advantageously less than 50 ppm and especially less than 20 ppm, for example between 1 and 5 ppm. For example, for the meglumine salt of gadolinium DOTA, a limit of 15 µg of Ca/g of DOTA powder (15 ppm) used in step b) corresponds to 3 µg Ca/ml of liquid contrast product administered to the patient (there is about 0.202 g of DOTA per ml of administered liquid contrast product), i.e. 3 ppm in this contrast product.

[0079] The different variants of the process according to the invention as described previously thus advantageously comprise, before the measuring and adjustment steps c) and d), an intermediate step b2) of controlling the amount of calcium in the formulations obtained in step b).

[0080] Where appropriate, in particular if the amount of calcium in the final solution is greater than 15 ppm or advantageously greater than 10 ppm, this intermediate step comprises, following this control, the removal of the excess calcium.

[0081] Thus, according to one aspect, the process according to the present invention is characterized in that the amount of calcium in the liquid pharmaceutical formulation administered to the patient is less than 50 ppm, especially less than 20 ppm, and preferably less than 5 ppm, the process advantageously comprising, before step c), an intermediate step b2) of measuring the amount of calcium and, where appropriate, of removing the excess calcium.

[0082] Furthermore, the different variants of the process according to the invention as described previously advantageously comprise, before step b), control of the amount of calcium in the components used in step b), and especially in the macrocyclic chelate intended to be dissolved, in the lanthanide (typically used in oxide form), and in the water. Advantageously, the amount of calcium in these components is less than 150 ppm and preferably less than 15 ppm. Thus, according to one aspect, the process according to the present invention is characterized in that the amount of calcium in these components (typically DOTA powder, gadolinium Gd₂O₃ powder, water) is less than 150 ppm and preferably less than 15 ppm. According to an aspect the invention concerns a DOTA as an intermediate product (DOTA powder or DOTA in aqueous solution) containing calcium at less than 150 ppm, preferably less than 50 ppm, and preferably less than 15 ppm.

[0083] Very advantageously, the Applicant has succeeded in removing the excess calcium in the chelate (powder) used in step b), by means, for DOTA, of a purification by crystallization using a water-ethanol mixture, which makes it possible to obtain an amount of calcium advantageously less than 50 ppm. The water used for step b) is also advanta-

geously purified, where appropriate by means of a suitable treatment, for example descaling with acids to prevent any undesired amount of calcium.

[0084] A gadolinium oxide with a purity very close to 100%, substantially of 99.999%, will preferably be used in particular.

[0085] Furthermore, it will be preferred to check that the meglumine used at the end of the adjustment step d) also comprises a small amount of calcium.

[0086] The process is also advantageously such that it uses components that have extremely low amounts of metals (for example nickel and aluminium) liable to interact with the chelate, disrupting the assays. Thus, the process advantageously includes a step of checking the amount of these metals before the measuring and adjustment steps b) and/or c) and/or d).

[0087] Finally, the process according to the present invention also advantageously comprises an additional step e) of checking C_{chl} and C_{lanl} , irrespective of the variant described above.

[0088] The process according to the present invention is, according to one preferred embodiment, characterized in that the pharmaceutical formulation is a pharmaceutical formulation of meglumine salt of the DOTA-gadolinium complex. The Applicant's process makes it possible to obtain the target formulations safely. This process makes it possible to solve the problem represented by the *in situ* complexation, in a pharmaceutical manufacturing reactor (into which is added the pharmaceutical formulation agent). Specifically, when the lanthanide is Gd^{3+} , meglumine will be used as formulation agent. However, given the physicochemical characteristics of gadoteric acid, the mixing of the three components (powder of non-complexed chelate, lanthanide powder and meglumine powder) in the same reactor would not be sufficiently satisfactory. Thus, the process according to the invention that allows this problem to be solved consists in engaging the complexation, measuring the difference relative to the target, and adjusting.

[0089] Overall, the Applicant's process thus makes it possible to incorporate the chelation process into the pharmaceutical production, with an advantage especially in terms of cost price and quality.

[0090] In one advantageous embodiment, an agent for blocking the free lanthanide, other than the free macrocyclic chelate, is added in step b). Advantageously, this blocking agent is a polycarboxylic acid, especially a dicarboxylic, tricarboxylic or tetracarboxylic acid, in particular a citrate or a derivative thereof.

[0091] As regards the general inventive concept of the target range of amount of free macrocyclic chelate (0.002% to 0.4%, advantageously 0.02% to 0.3% and in particular 0.025% to 0.25%), the Applicant points out that this range differs from the teaching of patent US 5 876 695 illustrated in particular by its examples, at least for the following reasons.

[0092] The Applicant's target range is very narrow, and corresponds to a selection within the very broad range presented in the said document.

[0093] The formulations described in US 5 876 695, which concern macrocyclic chelates (especially Examples 3 and 4), are formulations with salts of chelate (calcium disodium, zinc disodium DOTA) and not with free chelate. The amounts of excess salts therein are moreover very high, at least 10%. However, in the present patent application, only the free chelates are used, and not in the form of salts.

[0094] The formulations presented in US 5 876 695, which have an amount of free chelate of about 0.1%, concern only linear chelates (DTPA), and the DTPA formulation at 0.08% described is clearly indicated as a control solution, the said document suggesting, on the contrary, the use of a much higher amount, 2% or quite probably more.

[0095] Specifically, the only test presented as regards tolerance on the use of chelates, in Table 2 and in column 6 (lines 62-67) of the said document, shows that the reduction in toxicity is markedly less favourable for an amount of linear free chelate of 0.08 mol/mol% (Formulation A for which the amount is established on the basis of the ratio between 0.5 mmol Gd DTPA and 0.0004 mmol DTPA/kg), in comparison with the 2% amount corresponding to the advantageous formulation (Formulation B for which the amount established on the basis of the ratio between 0.5 mmol Gd DTPA and 0.01 mmol DTPA/kg) and which is described as a low value (column 6, line 61).

[0096] The Applicant thus worked on formulations with an amount of free macrocyclic chelate about 5 to 100 times lower than that explicitly recommended by document US 5 876 695. It was thus demonstrated by the Applicant, surprisingly, that macrocyclic chelates, and more especially DOTA, behave differently from linear chelates such as DTPA as regards tolerance, resulting from the presence of an excess of free chelate.

[0097] More specifically, whereas the tolerance appears to be improved with DTPA by increasing the excess free chelate from 0.08% to 2%, the tolerance degrades, in contrast, for DOTA by increasing the excess free chelate, passing from very low values (0.025% to 0.25%) to a value of 2%. Consequently, the transposition of values to reduce the risk of toxicity, between a linear chelate (in particular DTPA), and macrocyclic chelates (DOTA), is not at all obvious. This is moreover what is illustrated by the current complex discussions in the scientific community in the context of NSFs with regard to the tens of millions of doses of contrast agents already injected in man, discussions on the subject of the complexation kinetics and/or on comparisons of structures between chelates. For instance it has recently been shown that in order to reduce the risk of NSF (results on human skin where gadolinium accumulates) for some linear chelates, it is highly recommended to use very high quantities of excipient X,X'L for linear chelates, namely about 5 to 10% of such excipient, and that free chelate such as DTPA-BMA should clearly not be used.

[0098] To this end, the present description relates to a pharmaceutical formulation that may be obtained via the process

according to the present invention, characterized in that it contains between 0.002 and 0.4 mol/mol%, more especially between 0.02 and 0.3 mol/mol% and very advantageously between 0.025 and 0.25 mol/mol%, of free macrocyclic chelate (free DOTA).

[0099] By virtue of the adopted selection of the range of excess free macrocyclic chelate, of free DOTA, a value of free lanthanide in solution, of gadolinium, of about from 10^{-10} M to 10^{-14} M at physiological pH, is obtained.

[0100] The concentration of complexed chelate in the formulation is typically between 1 μ M and 1 M, with an administered dose of about from 0.01 to 5 mmol/kg. The concentration of the injected formulation is typically about 0.5 M.

[0101] The process particularly advantageously relates to the preparation of the pharmaceutical formulation of the meglumine salt of the DOTA-gadolinium complex: the macrocyclic chelate and the free macrocyclic chelate are DOTA, the lanthanide is gadolinium, and the prepared salt is the meglumine salt.

[0102] Advantageously, the pharmaceutical formulation is characterized in that the macrocyclic chelate is DOTA and in that the formulation contains between 0.02 and 0.08 mol/mol% of free DOTA.

[0103] This lower range is liable to have several physiological advantages:

- limiting a risk of chelation for certain diseases of endogenous cations (for example zinc or copper) by the presence of an overly large excess of macrocyclic chelate,
- limiting the inhibition of metalloenzymes, especially ACE, with an impact on the regulation of arterial hypertension, for example,
- avoiding unfavourable medicinal interactions with metallic active principles: lithium, bismuth, platinum, etc.,
- avoiding disrupting the seric dosages of endogenous metals,
- avoiding medicinal interactions with active principles that are complexing, for example detoxifying (deferoxamine, cyclam, etc.).

[0104] In another advantageous embodiment, the pharmaceutical formulation according to the present invention is characterized in that the macrocyclic chelate is DOTA and in that the formulation contains between 0.15 and 0.25 mol/mol% of an excess amount of free DOTA.

[0105] This higher range is liable to have several physiological advantages:

- optimally limiting the amount of free gadolinium injected, the free gadolinium being a toxicity risk and possibly being involved in phagocytosis mechanisms associated with certain diseases,
- minimizing the *in vivo* transmetallation in pathological situations, especially transmetallation by iron (increase in seric iron).

[0106] This higher range is also an advantage for further improving the stability of the formulation to be injected over time (dechelation under unsuitable storage conditions: heat, depressurization in aircraft, excessive exposure to light, etc.).

[0107] According to one embodiment, the amount of free macrocyclic chelate is between 0.09% and 0.15%. This median range is liable to combine advantages of the lower and higher ranges.

[0108] The choice of the amount of free macrocyclic chelate may be optimized in particular as a function of the risk of the patients for various pathologies or pathological risks associated with the mechanisms presented hereinabove. For example, in the case of patients presenting a risk of NSF, an excess of macrocyclic chelate in the median or high range may be preferred, to minimize any release of gadolinium.

[0109] Very low values of excess free chelate are, however, also liable to have a beneficial effect in the pathology NSF if it turns out in certain categories of patients (kidney failure patients in particular) that this pathology is partly associated with a presence of free chelate, which would involve *in vivo* transmetallation or similar phenomena that are unfavourable in terms of tolerance.

[0110] According to another aspect, the calcium content of the pharmaceutical formulation (administered to the patient) according to the invention is less than 50 ppm, advantageously less than 30 ppm and advantageously less than 15 ppm.

[0111] According to another aspect, the description relates to use of a contrast product formulation, the said formulation comprising a complex of macrocyclic chelate with a paramagnetic metal ion and an amount of free macrocyclic chelate of between 0.025% and 0.25%, advantageously of a formulation as mentioned above, for improvement of the tolerance.

[0112] According to another aspect, the description relates to a method for improving the *in vivo* tolerance of an MRI contrast product based on macrocyclic chelate, (on DOTA), which consists in using an excess of free chelate in an amount of between 0.025 and 0.25 mol/mol%, especially 0.025-0.08%, 0.09-0.15%, 0.16-0.25%.

[0113] Advantageously, the concentration of chelate (complexed chelate) in the formulation is between 0.5 and 0.9 M.

[0114] The macrocyclic chelate that is useful in the context of the present invention is DOTA, and derivatives thereof. The chemical formulae of these chelates are widely known to those skilled in the art, and are recalled, for example, in WO 2007/042 504, on pages 20 to 23, and WO 2003/011 115, on pages 8 to 11.

[0115] The description also relates to the use of a pharmaceutical formulation as mentioned above for the preparation

of a diagnostic composition for medical imaging, or for diagnostic monitoring of the efficacy of a therapeutic treatment, and to a diagnostic method comprising the administration of a pharmaceutically acceptable amount of a formulation as mentioned above.

[0116] For diagnosis in MRI, the intravenous administration by injection usually as a saline solution is typically performed at a dose of from 1 to 500 $\mu\text{mol Gd/kg}$. The pharmaceutically acceptable unit doses will depend on the nature of the chelate, the route of administration, and on the patient and especially on the nature of the disorder to be studied. For an intravenous injection and observation by magnetic resonance, the concentration of the solution will typically be between 0.001 and 0.5 mol/litre, and from 0.001 to 0.1 millimol/kg will be administered to the patient, depending on the case. Higher clinical doses may also be practised, for example a triple dose (0.3 millimol/kg). The administration rate, the concentration, the speed of injection are adapted according to the clinical indication and product specifications, and eventually also in view of the behaviour of the contrast agent during the MRI procedure. Any appropriate protocol is used, with possible adjustment of the administration in view of the patient data, of first test injections operated, of the enhancement curves obtained. The speed of injection may be calculated (advantageously automatically by data treatment tools) according to the protocol and during the protocol in view of the relaxivity curve during the course of the acquisition ; for instance if the administration rate/speed is not sufficient for optimal enhancement considering the data base, the injector automatically increases this rate during the MRI procedure.

[0117] Among the advantageous diagnostic indications, mention will be made of the indications already used clinically, and the indications for which the results are improved by virtue of the formulations according to the invention. Mention will thus be made of the following indications and improvements thereof: angiography, cerebral imaging, vascular imaging, imaging of cardiovascular, cancer, neurodegenerative and inflammatory pathologies, any indication with perfusion imaging, any indication combining the use of several contrast products, especially MRI, X-ray scanner, SPECT, PET, PET CT, and any indication with successive administrations of contrast products at the same or at different concentrations, or in multimodal imaging.

[0118] According to embodiments, these novel formulations may be chosen to be administered in combination with or in place of prior-art formulations as a function of the diagnostic profile of the patient, and especially of the profile of tolerance of the patient to the contrast products. The choice may be made by the practitioner and/or automatically by any tagging system (RFID tag carried by the patient, ...) and conditioning the type of administration, for example the choice of the contrast agent best adapted such as the formulation of the present application.

[0119] An installation comprising a device for evaluating the tolerance of the patient, and a device like an injector for administering the formulation of the contrast product as a function of the result given by the evaluation device may thus be used. Several risks may be evaluated, especially the risk of NSF (nephrogenic fibrosis). Where appropriate, the MRI product is co-administered simultaneously with or subsequently to at least one anti-NSF therapeutic agent (anti-fibrosis agent known therapeutically, especially steroids, anti-inflammatories or vitamins, for example). Where appropriate, an evaluation of the patient's risk with respect to NSF is performed to optimize the dose/concentration of injected contrast product (for example, the dose may be reduced relative to the common clinical dose, if it makes it possible, while avoiding any risk, to obtain sufficiently satisfactory information to obtain the signal in imaging).

[0120] To further reduce the risk of toxicity of the lanthanide in the case of at-risk patients, the Applicant also studied formulations comprising:

- as in the prior art: the chelate of lanthanide (for example gadoteric acid DOTA-Gd complex or a linear Gd-chelate) and the salification agent for neutralizing the chelate, for example meglumine (organic base),
- but in addition with at least one biocompatible supplementary excess blocking agent, intended to block any lanthanide (Gd^{3+}) that might otherwise remain free in the formulation.

[0121] Among the blocking agents that will especially be used are organic anions such as monocarboxylic or polycarboxylic acids (advantageously tricarboxylic or tetracarboxylic, such as citrate and derivatives thereof), hydroxy acids (lactate, malate ...), or other agents capable of an advantageous coordination interaction with the lanthanide.

[0122] The blocking agent may thus be introduced into the formulation and/or co-administered to the patient.

DETAILED EXAMPLES

1) Example 1: *In vivo* tolerance

[0123] The tolerance results in Table 2 (acute toxicity in mice for a diagnostic solution of DOTA ; this solution is a pharmaceutical solution injected and comprising the complex of DOTA with the Gd^{3+} , and an excess of free DOTA not complexed by Gd^{3+} and not complexed by metal ions as excipient) show that formulations containing from 0.025 to 0.25 mol/mol% of free macrocyclic chelate DOTA are three times less toxic than the formulation close to 2%.

EP 2 799 090 B9

Test	Excess of Free DOTA mol/mol %	Male LD ₅₀ Mmol/kg	Female LD ₅₀ Mmol/kg
1	0.05	12.41	13.59
2	0.09	13.06	13.50
3	0.25	12.02	12.07
4	1.98	4.80	4.80

[0124] Further stability studies performed by the Applicant show that the formulations are very satisfying with no release of gadolinium for a long conservation time.

Reference Example 2: Process for preparing formulations of lanthanide chelate (mixture of a solution of chelate and of a solution of lanthanide)

[0125] The preparation of formulations in which the macrocyclic chelate is DOTA is more specifically described. Table 3 below gives an example of the amounts used for the manufacture of a solution of 100 litres of DOTA (industrial amount).

<i>Component</i>	<i>Amount</i>
DOTA (1)	20.100 kg (i.e. 0.497 M)
Gadolinium oxide (expressed as anhydrous product)	9.135 kg (i.e. 0.504 M)
Meglumine (expressed as anhydrous product)	9.215 kg
Solution for adjusting DOTA to 5% qs amount of free DOTA	15-35 mg per 100 ml
3N meglumine solution qs pH=6.8-7.4 at 20°C	
Injection-grade water qs	100 litres
(1) 1,4,7,10-Tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid	

Step 1: dissolution

[0126] 40 litres of injection-grade water at 80°C are placed in a 100-litre manufacturing tank, the injection of nitrogen is started, and the 20.100 kg of DOTA and the 9.135 kg of gadolinium oxide are then incorporated with stirring. The complexation is performed at a pH below 6, for example between 3 and 6, for example at pH 4. The gadolinium oxide in the presence of DOTA forms a water-soluble acid complex.

Step 2: measurements

[0127] After step 1, a sample is taken and the free gadolinium is assayed.

Step 3: adjustment of the free species

[0128] The adjustment of the solution is advantageously performed with gadolinium oxide or DOTA.

[0129] A DOTA-adjusting solution is thus added qs an amount of 15-35 mg per 100 ml.

Step 4: cooling

[0130] The final solution from step 3 is cooled to 30°C, for example by circulating cold water in the tank jacket.

Step 5: adjustment of the pH and of the mass per unit volume

[0131] The acid function of the complex formed is salified with meglumine and the pH at 20°C is adjusted to 6.8 - 7.4. The concentration is adjusted by adding injection-grade water.

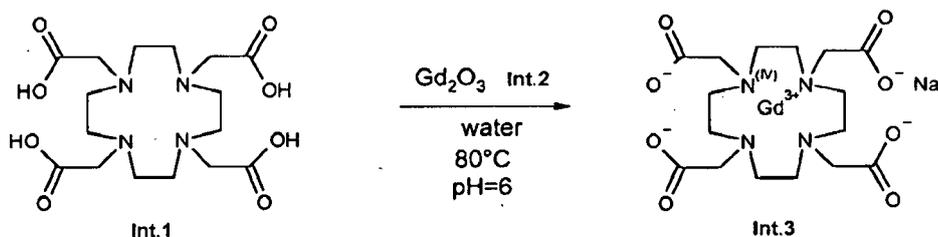
[0132] The following are thus introduced into the manufacturing tank:

- 9.125 kg of meglumine
- and a solution of meglumine pH = 6.8-7.4 at 3N
- injection-grade water, qs.

5 **[0133]** The final solution is then filtered and then placed in bottles typically sterilized by autoclaving.

2) Example 3: Process for preparing formulations of lanthanide chelate (dissolution of a solid complex [chelate-lanthanide])

10 **[0134]**



20 **[0135]** This example illustrates the manufacture of a small amount of product, the appropriate transposition being performed at the industrial scale.

	Int.1	Int.2 (Gd ₂ O ₃)	Int.3
Mw (g.mol ⁻¹)	404.42	362.70	580.63
m (g)	10	4.48	
n (mol)	0.025 (1 Eq)	0.0125 (0.5 Eq)	

30 **[0136]** 10 g (0.025 mol; 1 eq) of macrocyclic chelate DOTA are dissolved in 200 ml of water by heating to 80°C, in a three-necked flask equipped with a condenser, a thermometer and a pH meter. The measured pH is 3.7. It is adjusted to 6 with 2N NaOH solution. 4.48 g (0.0125 mol; 0.5 eq) of gadolinium oxide are added. The pH is readjusted and kept stable at between 6 and 7 by adding 1N HCl. The reaction is left at 80°C with stirring.

35 **[0137]** The residual free gadolinium is removed by means of a chelex resin prerinsed with water. To do this, the reaction mixture is brought to pH 5 (the resin is more efficient). The whole is left for 2 hours with stirring at room temperature. The pH rises to between 6.5 and 7. The resin is removed by filtration.

[0138] The complex is precipitated in ethanol to remove the salts (5 volumes of EtOH per 1 volume of water).

40 **[0139]** An assay of the salts is performed by titration with a 0.05N silver nitrate solution. Quantification of the free gadolinium is also performed by colorimetric assay with Arsenazo (III). 11.5 g of product are obtained (white powder). Yield = 80%; HPLC purity: 98%; LC/MS (ES⁺ mode): z = 1 (m/z = 559).

[0140] The dissolution in water is then performed via suitable methods, for example using a water at 45°C, with stirring for about 30 minutes, and with adjustment of the pH.

45

Claims

1. Process for preparing a liquid pharmaceutical formulation containing a complex of macrocyclic chelate with a lanthanide and a mol/mol amount of free macrocyclic chelate of between 0.002% and 0.4%, said process comprising the following successive steps:

55 b) preparation of a liquid pharmaceutical composition containing the complex of macrocyclic chelate with a lanthanide, free macrocyclic chelate that is not under the form of an excipient X[X',L] in which L is the macrocyclic chelate and X and X' are a metal ion, in particular chosen independently from calcium, sodium, zinc and magnesium, and/or free lanthanide, said step b) consisting in the preparation of a solid complex [chelate-lanthanide] and in the dissolution of the said complex in water ;

- c) measurement in the pharmaceutical formulation obtained in step b) of the concentration of free macrocyclic chelate C_{chl} and/or of free lanthanide C_{lanl} ;
 d) adjustment of C_{chl} and/or of C_{lanl} by adding to the formulation obtained in step b) :

- 5
 - if $C_{lanl} > 0$ and/or $C_{chl} < C_{tchl}$, free macrocyclic chelate,
 - if $C_{lanl} = 0$ and $C_{chl} > C_{tchl}$, free lanthanide,

so as to obtain $C_{chl} = C_{tchl}$ and $C_{lanl} = 0$ with C_{tchl} representing the target concentration of the free macrocyclic chelate in the final liquid pharmaceutical formulation, C_{tchl} being selected in the range of between 0.002 % and 0.4 % mol/mol,

10
 wherein the amount of free macrocyclic chelate in the final liquid pharmaceutical formulation corresponds to the proportion of free macrocyclic chelate relative to the amount of complexed macrocyclic chelate DOTA-Gd in the final liquid pharmaceutical formulation in mol/mol,
 and wherein the macrocyclic chelate is DOTA and the lanthanide is gadolinium.

- 15
 2. Process according to claim 1, **characterized in that** the mol/mol amount of free macrocyclic chelate of between 0.025% and 0.25%.
- 20
 3. Process according to claim 1 or 2, **characterized in that** the pharmaceutical formulation is a pharmaceutical formulation of the meglumine salt of a DOTA-gadolinium complex.
- 25
 4. Process according to claim 3, **characterized in that** the adjustment step d) comprises at the end a step of adjustment of the pH and of the volume, with meglumine.
- 30
 5. Process according to any of claims 1 to 4, **characterized in that** the amount of calcium in the liquid pharmaceutical formulation to be administered to the patient is less than 50 ppm, advantageously less than 20 ppm, more advantageously less than 5 ppm.
- 35
 6. Process according to claim 5, **characterized in that** the amount of calcium in the liquid pharmaceutical formulation to be administered to the patient is less than 50 ppm and **in that** the amount of calcium in the ingredients used for the pharmaceutical solution in step b), namely the DOTA powder, water and meglumine, is less than 50 ppm, advantageously less than 20 ppm.
- 40
 7. Process according to any of claims 5 or 6, **characterized in that** it comprises, before step c), an intermediate step b2) of measuring the amount of calcium and, where appropriate, of removing the excess calcium.
- 45
 8. Process according to any of claims 1 to 7, **characterized in that** it comprises an additional step e) of checking C_{chl} and C_{lanl} .
9. Process according to claim 1, **characterized in that** the preparation of the said solid complex involves at least one treatment step chosen from filtration, concentration, crystallization, drying and spraying, for obtaining the appropriate physicochemical characteristics in terms of solubility and purity.
10. Process according to any of claims 1 to 9, **characterized in that** step c) is carried out at pH 7.

Patentansprüche

- 50
 1. Verfahren zum Herstellen einer flüssigen pharmazeutischen Formulierung, die einen Komplex von makrocyclischem Chelat mit einem Lanthanid und einer mol/mol-Menge an freien makrocyclischen Chelat zwischen 0,002% und 0,4% enthält, wobei das Verfahren folgende aufeinanderfolgende Schritte umfasst:
- 55
 b) Herstellen einer flüssigen pharmazeutischen Zusammensetzung, die den Komplex von makrocyclischem Chelat mit einem Lanthanid, freies makrocyclisches Chelat, das nicht in der Form eines Hilfsstoffs $X[X',L]$ vorliegt, wobei L das makrocyclische Chelat ist und X und X' ein Metallion, insbesondere unabhängig ausgewählt aus Calcium, Natrium, Zink und Magnesium, sind, und/oder freies Lanthanid enthält, wobei Schritt b) aus der Herstellung eines festen Komplexes [Chelat-Lanthanid] und dem Lösen des Komplexes in Wasser besteht;

EP 2 799 090 B9

- c) Messen der Konzentration an freiem makrocyclischen Chelat C_{chI} und/oder freiem Lanthanid C_{lanI} in der in Schritt b) erhaltenen pharmazeutischen Formulierung;
d) Einstellen von C_{chI} und/oder C_{lanI} durch Zugabe von:

- 5 - wenn $C_{lanI} > 0$ und/oder $C_{chI} < C_{tchI}$, freiem makrocyclischen Chelat,
 - wenn $C_{lanI} = 0$ und $C_{chI} > C_{tchI}$, freiem Lanthanid, zu der in Schritt b) erhaltenen Formulierung,

so dass man $C_{chI} = C_{tchI}$ und $C_{lanI} = 0$ erhält, wobei C_{tchI} die Zielkonzentration des freien makrocyclischen Chelats in der fertigen flüssigen pharmazeutischen Formulierung ist, wobei C_{tchI} aus dem Bereich zwischen 0,002% und 0,4% mol/mol gewählt ist,
wobei die Menge an freiem makrocyclischen Chelat in der fertigen flüssigen pharmazeutischen Formulierung dem Anteil an freiem makrocyclischen Chelat bezogen auf die Menge an komplexiertem makrocyclischen Chelat DOTA-Gd in der fertigen flüssigen pharmazeutischen Formulierung in mol/mol entspricht,
und wobei es sich bei dem makrocyclischen Chelat um DOTA und bei dem Lanthanid um Gadolinium handelt.

- 15 **2.** Verfahren nach Anspruch 1, **dadurch gekennzeichnet, dass** die mol/mol-Menge an freiem makrocyclischen Chelat zwischen 0,025% und 0,25% liegt.
- 20 **3.** Verfahren nach Anspruch 1 oder 2, **dadurch gekennzeichnet, dass** es sich bei der pharmazeutischen Formulierung um eine pharmazeutische Formulierung des Megluminsalzes eines DOTA-Gadolinium-Komplexes handelt.
- 4.** Verfahren nach Anspruch 3, **dadurch gekennzeichnet, dass** der Einstellungsschritt d) am Ende einen Schritt der Einstellung des pH-Wertes und des Volumens mit Meglumin umfasst.
- 25 **5.** Verfahren nach einem der Ansprüche 1 bis 4, **dadurch gekennzeichnet, dass** die Menge an Calcium in der dem Patienten zu verabreichenden flüssigen pharmazeutischen Formulierung weniger als 50 ppm, vorteilhaft weniger als 20 ppm, besonders vorteilhaft weniger als 5 ppm beträgt.
- 30 **6.** Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** die Menge an Calcium in der dem Patienten zu verabreichenden flüssigen pharmazeutischen Formulierung weniger als 50 ppm beträgt und dass die Menge an Calcium in den für die pharmazeutische Lösung in Schritt b) verwendeten Inhaltsstoffen, nämlich dem DOTA-Pulver, Wasser und Meglumin, weniger als 50 ppm, vorteilhaft weniger als 20 ppm beträgt.
- 7.** Verfahren nach Anspruch 5 oder 6, **dadurch gekennzeichnet, dass** es vor Schritt c) einen Zwischenschritt b2) umfasst, bei dem man die Menge an Calcium misst und gegebenenfalls überschüssiges Calcium entfernt.
- 35 **8.** Verfahren nach einem der Ansprüche 1 bis 7, **dadurch gekennzeichnet, dass** es einen zusätzlichen Schritt e) umfasst, bei dem man C_{chI} und C_{lanI} überprüft.
- 40 **9.** Verfahren nach Anspruch 1, **dadurch gekennzeichnet, dass** die Herstellung des festen Komplexes mindestens einen aus Filtrieren, Konzentrieren, Kristallisieren, Trocknen und Sprühen ausgewählten Behandlungsschritt umfasst, so dass man die entsprechenden physikochemischen Charakteristika im Hinblick auf Löslichkeit und Reinheit erhält.
- 45 **10.** Verfahren nach einem der Ansprüche 1 bis 9, **dadurch gekennzeichnet, dass** man Schritt c) bei einem pH-Wert von 7 durchführt.

Revendications

- 50 **1.** Procédé de préparation d'une formulation pharmaceutique liquide contenant un complexe d'un chélate macrocyclique avec un lanthanide et une quantité mol/mol de chélate macrocyclique libre comprise entre 0,002 % et 0,4 %, ledit procédé comprenant les étapes successives suivantes :

55 b) préparation d'une composition pharmaceutique liquide contenant le complexe du chélate macrocyclique avec un lanthanide, le chélate macrocyclique libre qui n'est pas sous la forme d'un excipient $X[X',L]$ dans lequel L et le chélate macrocyclique et X et X' représentent un ion métallique, en particulier choisi indépendamment parmi le calcium, le sodium, le zinc et le magnésium, et/ou un lanthanide libre,

ladite étape b) consistant en la préparation d'un complexe solide [chélate-lanthanide] et en la dissolution dudit complexe dans l'eau ;

c) mesure dans la formulation pharmaceutique obtenue à l'étape b) de la concentration de chélate macrocyclique libre C_{chl} et/ou de lanthanide libre C_{lanl} ;

d) ajustement de C_{chl} et/ou de C_{lanl} par ajout à la formulation obtenue dans l'étape b) :

- si $C_{lanl} > 0$ et/ou $C_{chl} < C_{tchl}$, de chélate macrocyclique libre,
- si $C_{lanl} = 0$ et $C_{chl} > C_{tchl}$, de lanthanide libre,

de sorte à obtenir $C_{chl} = C_{tchl}$ et $C_{lanl} = 0$ avec C_{tchl} représentant la concentration cible du chélate macrocyclique libre dans la formulation pharmaceutique liquide finale, C_{tchl} étant choisie dans la plage comprise entre 0,002 % et 0,4 % mol/mol,

où la quantité de chélate macrocyclique libre dans la formulation pharmaceutique liquide finale correspond à la proportion de chélate macrocyclique libre par rapport à la quantité de chélate macrocyclique complexé DOTA-Gd dans la formulation pharmaceutique liquide finale en mol/mol, et où le chélate macrocyclique est le DOTA et le lanthanide est le gadolinium.

2. Procédé selon la revendication 1, **caractérisé en ce que** la quantité mol/mol de chélate macrocyclique libre est comprise entre 0,025 % et 0,25 %.
3. Procédé selon la revendication 1 ou 2, **caractérisé en ce que** la formulation pharmaceutique est une formulation pharmaceutique du sel de méglumine d'un complexe DOTA-gadolinium.
4. Procédé selon la revendication 3, **caractérisé en ce que** l'étape d'ajustement d) comprend à la fin une étape d'ajustement du pH et du volume avec de la méglumine.
5. Procédé selon l'une quelconque des revendications 1 à 4, **caractérisé en ce que** la quantité de calcium dans la formulation pharmaceutique liquide à administrer au patient est inférieure à 50 ppm, avantageusement inférieure à 20 ppm, plus avantageusement inférieure à 5 ppm.
6. Procédé selon la revendication 5, **caractérisé en ce que** la quantité de calcium dans la formulation pharmaceutique liquide à administrer au patient est inférieure à 50 ppm et **en ce que** la quantité de calcium dans les ingrédients utilisés dans la solution pharmaceutique de l'étape b), nommément la poudre de DOTA, l'eau et la méglumine, est inférieure à 50 ppm, avantageusement inférieure à 20 ppm.
7. Procédé selon l'une quelconque des revendications 5 ou 6, **caractérisé en ce qu'il** comprend, avant l'étape c), une étape intermédiaire b2) de mesure de la quantité de calcium et, lorsque cela est approprié, d'élimination du calcium en excès.
8. Procédé selon l'une quelconque des revendications 1 à 7, **caractérisé en ce qu'il** comprend une étape supplémentaire e) de vérification de C_{chl} et de C_{lanl} .
9. Procédé selon la revendication 1, **caractérisé en ce que** la préparation dudit complexe solide implique au moins une étape de traitement choisie parmi une filtration, une concentration, une cristallisation, un séchage et une pulvérisation, pour l'obtention des caractéristiques physicochimiques appropriées en termes de solubilité et de pureté.
10. Procédé selon l'une quelconque des revendications 1 à 9, **caractérisé en ce que** l'étape c) est mise en oeuvre à pH 7.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 4647447 A [0002]
- US 5876695 A [0004] [0005] [0012] [0033] [0076] [0091] [0093] [0094] [0096]
- EP 454078 A [0007]
- US 7385041 B [0007] [0008] [0012]
- WO 2007106544 A [0013]
- US 5082649 A [0076]
- WO 2007042504 A [0114]
- WO 2003011115 A [0114]

Non-patent literature cited in the description

- **COROT et al.** *Journal of Magnetic Resonance Imaging*, May 1998, vol. 8 (3), 695-702 [0010]
- *Chem. Eur. J.*, 2004, vol. 10, 5218-5232 [0068]