Description

The present invention relates to formulations for radiopharmaceuticals, their method of administration and process of preparation. Radiopharmaceutical formulations have several applications in the therapeutic and/or diagnostic treatment of animals for various diseases, especially for cancer.

The development of bone metastasis is a common and often catastrophic event for a cancer patient. The pain, pathological fractures, frequent neurological deficits and forced immobility caused by these metastatic bone lesions significantly decrease the quality of life for the cancer patient. The number of patients that contract metastatic disease is large since nearly 50 percent of all patients who contract breast, lung or prostate carcinoma will eventually develop bone metastasis. Bone metastasis are also seen in patients with carcinoma of the kidney, thyroid, bladder, cervix, and other tumors, but collectively, these represent less than 20 percent of patients who develop bone metastasis. Metastatic bone cancer is rarely life threatening and occasionally patients live for years following the discovery of the bone lesions. Initially, treatment goals center on relieving pain, thus reducing requirements for narcotic medication and increasing ambulation. Clearly, it is hoped that some of the cancers can be cured.

Radiopharmaceuticals used in the formulations of the present invention have been prepared as their metal-ligand complexes, particularly the agent samarium-153-ethylenediaminetetramethylenephosphonic acid ("^{153}Sm-EDTMP") as disclosed in U.S. Patent 4,898,724. The ^{153}Sm-EDTMP, especially as its pharmaceutical formulation, has a utility for the relief of bone pain and for the treatment of calcific tumors, which utility and preparation has been given in U.S. Patent 4,898,724, Canadian Patent 1,243,603, and published European Application 164,843, The additional use of radiopharmaceuticals for bone marrow suppression is given in U.S. Patent 4,853,209. All of these references disclose a use of the radiopharmaceuticals of the present invention, particularly ^{153}Sm-EDTMP, in a formulation with suitable pharmaceutically-acceptable carriers.

One concern when administering any radiopharmaceutical is the potential for radiolytic degradation of the organic(s) molecules present in the formulation which may alter the biodistribution of the radioisotope or result in toxic by-products. Neither of these events is desirable. When high amounts of radioactivity are needed, there is the increased potential for radiation damage to the organic molecule (e.g. EDTMP). This degradation is more likely to occur when therapeutic radionuclides (e.g. ^{153}Sm) are used which are designed to deliver high radiation doses.

One approach that has been tried to prevent radiolysis is to add a free radical inhibitor to the formulation. However, the inhibitor or its degradation products may be toxic or interfere with the biodistribution of the radiopharmaceutical. Use of an inhibitor such as benzyl alcohol is discussed by H. Ikebuchi et al., *Radioisotopes* 26(7), 451-7 (1977); B. J. Floor et al., *J.Pharm.Sci.* 74(2), 197-200 (1985); and A. Rego et al., *J.Pharm.Sci.* 71(11), 1219-23 (1982).

A further concern for radiopharmaceutical formulations (as shown in U.S. Patent 4,898,724 and published European Application 164, 843) is that a molar excess of ligand (e.g. EDTMP) compared with the amount of metal may be used. When large amounts of metal ions (e.g. Sm^{+3}) are injected, a much larger amount of free chelating agent (e.g. EDTMP) is therefore also injected. The excess ligand present may be able to complex metal ions in the bloodstream which may lead to complications for the patient. It is therefore desirable to have a formulation that contains a minimal amount of free chelating agent.

Another concern when administering radiopharmaceuticals is the mode of injection. Typically, all such radiopharmaceuticals are administered by intravenous injections (I.V.). The patient can experience some discomfort with an I.V. injection and sometimes it is difficult to find a suitable vein available in the patient.

Consequently, it would be advantageous to have a formulation for radiopharmaceuticals which had minimal radiolysis prior to use, could be injected by several routes, and avoided excess free (or uncomplexed) ligand.

The accompanying drawings are provided to illustrate radiolysis as follows:

Figure 1 shows the radiolysis of EDTMP with 100 mCi/mL specific activity of ^{153}Sm in the presence of 5 percent ethanol ("EtOH";+), 0.9 percent benzyl alcohol ("BzOH":◊), frozen (Δ) and a control (▮).

Figure 2 shows the radiolysis of EDTMP as uninhibited kits at different specific activities of ^{153}Sm: ▮ 100mCi/ml; + 50 mCi/ml; ◊ 30 mCi/ml; Δ 30 mCi/ml.

Surprisingly, an improved formulation for administering radiopharmaceuticals, especially ^{153}Sm-EDTMP, has now been found which reduces radiolysis of the EDTMP without altering the performance of the radiopharmaceutical. Radiopharmaceuticals suitable for use in the present formulations of this invention include complexes comprising at least one radionuclide complexed with a ligand or a physiologically-acceptable salt thereof.

In a first aspect of the present invention, there is provided a radiopharmaceutical formulation comprising a complex of Samarium-153, Holmium-166, Ytterbium-175, Lutetium-177, Yttrium-90, or Gadolinium-159 with ethylenediaminetetramethylenephosphonic acid or a physiologically-acceptable salt thereof, characterized in that the formulation is frozen, and prior to use is thawed. Especially preferred radionuclides are ^{153}Sm and ^{166}Ho, with ^{153}Sm being most preferred.

A second aspect of the invention provides a radiopharmaceutical formulation comprising a complex of a radionuclide with a ligand, or a physiologically-acceptable salt thereof, characterized in that the formulation also comprises a divalent metal.

The two aspects of the invention may or may not be used simultaneously.

The radionuclides employed in the first aspect of the invention are Samarium-153 (^{153}Sm), Holmium-166 (^{166}Ho), Ytterbium-175 (^{175}Yb), Lutetium-177 (^{177}Lu), Yttrium-90 (^{90}Y), or Gadolinium-159 (^{159}Gd), and, as indicated above, these may also advantageously be employed in the second aspect of the invention. Especially preferred radionuclides are ^{153}Sm and ^{166}Ho, with ^{153}Sm being most preferred.

Examples of suitable ligands for use in the second aspect of the invention are ethylenediaminetetramethylenephosphonic acid ("EDTMP"), diethylenetriaminepentamethylenephosphonic acid ("DTPMP"), hydroxyethylethylenediaminetrimethylenephosphonic acid ("HEEDTMP"), nitrilotrimethylenephosphonic acid ("NTMP"), tris(2-aminoethyl)aminehexamethylenephosphonic acid ("TTHMP"), 1-carboxyethylenediaminetetramethylenephosphonic acid ("CEDTMP"), bis-(aminoethylpiperazine)-tetramethylenephosphonic acid ("AEPTMP"), and 1,4,7,10-tetraazacyclododecanetetramethylenephosphonic acid ("DOTMP"), and physiologically-acceptable salts thereof. Particularly preferred ligands are EDTMP, DTPMP, HEEDTMP, TTHMP, AEPTMP, CEDTMP and DOTMP, with EDTMP being most preferred. A particularly preferred radiopharmaceutical for use in the present invention is ^{153}Sm-EDTMP and its physiologically-acceptablesalts.

The present formulations can also contain a divalent metal ion of from 0.25 to 5 moles of divalent metal ion per mole of ligand which does not interfere with the formation of the radiopharmaceutical complex. When the divalent metal ion is added prior to the radionuclide, the formulation does not require freezing until the addition of the radionuclide occurs and then only if a significant time lapses between the formation of the radiopharmaceutical formulation and its use.

Also, it has now been found that intravenous ("I.V.") injection is not required to obtain the desired biodistribution of radionuclide when using a radiopharmaceutical formulation of the present invention. Rather, when desired or necessary, intraperitoneal ("I.P."), subcutaneous ("S.C.") or intramuscular ("I.M.") injections provide similar biodistributions.

The present radiopharmaceutical formulations which contain the divalent metal minimize the presence of free (or uncomplexed) ligand (e.g., EDTMP) to be introduced into the bloodstream of the mammal. This divalent metal-ligand complex reduces the chelation of other metals (e.g., calcium) in the blood by the ligand. Thus, the deleterious effect of the excess ligand is decreased. However, the divalent metals must not interfere with the formation of the radiopharmaceutical complex (e.g., ^{153}Sm-EDTMP). Suitable divalent metals are Fe^{+2} and Mn^{+2} and the alkaline earths, for example, Mg^{+2}, Ca^{+2}, Sr^{+2} and Ba^{+2}, with Ca^{+2} being most preferred. These radiopharmaceutical formulations are prepared such that from 0.25 to 5 moles of the divalent metal is present per mole of ligand, especially preferred from 0.5 to 3 moles, preferably from 0.5 to 1 mole, more preferably from 0.75 to 1 mole, and most preferably from 0.9 to 1 mole.

There are several ways to prepare such divalent metal radiopharmaceutical formulations. One way (Method A) is by preparing a divalent metal formulation which is added to the radioactive metal-ligand complex. The radioactive metal-ligand complex may optionally have been frozen, then thawed to add the divalent metal and could, if desired, be refrozen and then thawed prior to use. A second way (Method B) is by adding the divalent metal to the radioactive metal ion solution. The ligand is then added to from the complex and then this formulation is frozen, if desired. Because radiolysis does not occur until the ligand is added, freezing is used if the time lapse prior to use makes that desirable. The third way, is to coadminister the radiopharmaceutical, which may have been frozen and thawed for use, with a divalent metal ion solution, e.g., calcium gluconate, by two separate I.V. injections at about the same time to the patient. The fourth way, and the preferred way (Method D) is to add the divalent metal, as its chloride or more preferably is its hydroxide, to the ligand and then adjust the pH. e.g., by adding sodium hydroxide ("NaOH"), optionally freeze-drying the solution to form a divalent metal-ligand lyophilized formulation (kit). The acidic solution of radioactive metal ion is used to reconstitute the kit and results in the radiopharmaceutical formulation suitable for use and at the desired pH. One concern in any of the methods to form the radiopharmaceutical formulation is the half-life of the radionuclide being used (e.g., ^{153}Sm $t_{1/2}$ = about 46 hours). Another concern is the length of time that the radionuclide is in solution with the ligand. The amount of activity and the time the radionuclide is in solution with the ligand will determine the desirability of

freezing the radiopharmaceutical formulation.

Kits were prepared containing a ligand formulation of about one equivalent of divalent metal per equivalent of ligand in the preferred embodiment (Method D). Also the divalent metal-ligand solution can be freeze-dried, which, when reconstituted with a solution of radionuclide, forms a quantitative complex with the radioactive metal ion and has a pH of from 7 to 8.5. The divalent metal formulation is especially preferred when large doses of ligand are used, usually with the higher doses of radionuclide, especially used for bone marrow ablation.

Thus, preferably the kits containing the ligand, such a EDTMP, optionally with the divalent metal present, are freeze-dried in such a manner that the addition of a predetermined amount of radionuclide solution in a suitable concentration of HCl (preferably from about 0.001 to 1N HCl, or more preferably from 0.01 to 0.1N HCl) which would result in a quantitative yield of radionuclide-ligand complex, e.g., Sm-EDTMP, and have a resulting pH of between 7 to 8.5. For the radiopharmaceutical formulation ^{153}Sm-EDTMP, the preferred concentration of EDTMP is 35 mg/mL and the preferred Sm concentration is 3 x 10^{-4}M (i.e. about 278:1 EDTMP:Sm).

The present radiopharmaceutical formulations, especially ^{153}Sm-EDTMP formulations, with or without the divalent metal present, can be frozen to minimize radiolysis of the ligand, then thawed prior or use. The freezing can be done by any convenient means which maintains the sterility of the product (e.g., by liquid nitrogen or dry ice), then when it is desired to use the formulation, it is allowed to thaw.

The route of administration of the present formulations may be selected from I.V., I.P., S.C. or I.M. The biodistribution results are similar for the present radiopharmaceutical formulations by all the injection routes.

An improved method for preparing radiopharmaceuticals, especially ^{153}Sm-EDTMP, has now been found which reduces radiolysis of the ligand without altering the performance of the radiopharmaceutical. Radiopharmaceuticals suitable for use in the present formulations of this invention include complexes comprising at least one radionuclide complexed with a ligand, or physiologically-acceptable salts thereof.

Examples of suitable radionuclides are Samarium-153 (^{153}Sm), Holmium-166 (^{166}Ho), Ytterbium-175 (^{175}Yb), Lutetium-177 (^{177}Lu), Yttrium-90 (^{90}Y) or Gadolinium-159 (^{159}Gd). Especially preferred radionuclides are ^{153}Sm and ^{166}Ho, with ^{153}Sm being most preferred.

Example of suitable ligands in the second aspect of the invention are ethylenediaminetetramethylenephosphonic acid "EDTMP"). diethylenetriaminepentamethylenephosphonic acid ("DTPMP"), hydroxyethylethylenediaminetrimethylenephosphonic acid ("HEEDTMP"), nitrilotrimethylenephosphonic acid ("NTMP"), tris(2-aminoethyl)aminehexamethylenephosphonic acid ("TTHMP"), 1-carboxyethylenediaminetetramethylenephosphonic acid ("CEDTMP"), bis-(aminoethylpiperazine)tetramethylenephosphonic acid (AEPTMP"), and 1,4,7,10-tetraazacyclododecanetetramethylenephosphonic acid ("DOTMP"), and physiologically-acceptable salts thereof. Particularly preferred ligands are EDTMP, DTPMP, HEEDTMP, TTHMP, AEPTMP, CEDTMP and DOTMP, with EDTMP being particularly preferred. A particularly preferred radiopharmaceutical for use in the present invention is ^{153}Sm-EDTMP and its physiologically-acceptable salts. Suitable physiologically-acceptable salts have been defined in U.S. Patent 4,898,724 and include the acid addition salts of those bases which will form a salt with at least one acid group of the ligand employed and which will not cause a significant adverse physiological effect when administered to an animal at dosages consistent with good pharmacological practice. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates, such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate, ammonia, primary, secondary and tertiary amines. These physiologically-acceptable salts may be prepared by contacting acid with the appropriate base.

Definitions

Sm = Samarium, all isotopes both radioactive and not. (Similarly for the other radionuclides of the invention.)

^{153}Sm = is the radioactive isotope of samarium having an atomic mass of 153. (Similarly for the other radionuclides of the invention.)

EDTMP = ethylenediaminetetramethylenephosphonic acid.

^{153}Sm-EDTMP = ^{153}samarium-ethylenediaminetetramethylenephosphonic acid, which is a complex in solution; it contains both radioactive and non-radioactive isotopes of samarium.

Spiked = 0.5 to 1 picoCi $^{153}SmCl_3$ or $^{166}HoCl_3$ solution was added as a tracer.

Radiopharmaceutical = a radioactive pharmaceutical for diagnosis and/or therapy, usually a solution containing a radioactive metal ion (e.g., ^{153}Sm) attached to an organic ligand (e.g., EDTMP) in an aqueous

solution.

Kit = a vial containing a solid ligand formulation, optionally containing a divalent metal ion, to which a solution of radioactive metal ion is added for the purpose of forming the radioactive metal-ligand complex.

X-mL kit = a kit designed to receive X-mL of radioactive metal ion solution for the purpose of forming the radionuclide-ligand complex.

Reconstituted = addition of an acidic radionuclide solution to a kit for the purpose of forming a radionuclide-ligand complex.

TBA = tetrabutylammonium hydroxide

BzOH = benzyl alcohol

EtOH = ethanol

% = percent

HCl = hydrochloric acid

NaOH = sodium hydroxide

I.V. = intravenous injection

I.P. = intraperitoneal injection

I.M. = intramuscular injection

S.C. = subcutaneous injection

MDP = methylenediphosphonate

General Experimental

The cation exchange chromatographic separation method was used to determine the complex yield. This procedure is described in U.S. Patent 4,898,724.

HPLC : high performance liquid chromatography; the column used was a Hamilton™ PRP-1 reverse phase, the eluent was 0.1M sodium acetate and 0.005M TBA. The flow rate was 1 mL/min; detection was with a radiometric detector and U.V. detector (240 nm) coupled in series.

The EDTMP was prepared in a pure form, suitable for use as a pharmaceutical by the method described in U.S. Patent 4,937,333 and published European Application 411,941.

The ^{153}Sm was obtained from the research reactor at the University of Missouri, Columbia, MO.

Barnstead NANOpure™ water was used as the distilled water.

$Ca(OH)_2$ was as the dihydrate from MCB or Aldrich, 95 percent pure.

All reagents not otherwise specified were purchased and used as received.

The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention.

Example 1: Method A, no calcium added

Three mL of a $8x10^{-2}M$ EDTMP solution was prepared that was $3x10^{-4}M$ in Sm. The specific activity was 100 mCi of ^{153}Sm per mL. Three 200 µL aliquots (Samples I, II and III were placed in plastic vials and frozen using a dry ice-acetone bath. The plastic vials were kept in a freezer. Another 500µL portion was placed in a glass vial and 4.3 µL of benzyl alcohol were added to result in a 0.9 percent (weight/volume) solution (Sample A). A further 500 µL portion was placed in a glass vial and 31.8 µL of ethanol were added to result in 5 percent (weight/volume) solution (Sample B).

The solution were analyzed by HPLC and degradation was monitored as a function of time. Degradation was evidence by the appearance of non-Sm-EDTMP peaks in the radiometric chromatogram. For this time study, one frozen plastic vial was allowed to thaw at ambient temperature and a portion was analyzed. The solution was allowed to stand without freezing for 2 to 4 hours and the analysis on the solution repeated. The other two plastic vials were used to get data at larger time intervals in a similar manner.

The result showed only one radiometric peak corresponding to the desired product for over a 60 hour period for the frozen samples. The control (non-inhibited sample) and both the benzyl alcohol and the ethanol samples showed several radiometric peaks. The percent radiation that did not correspond to the desired product as a function of time from reconstitution is shown in Table 1.

Table 1
INHIBITION OF RADIOLYTIC DEGRADATION
(100 mCi/mL)

Sample	Time (hours)	% Degradation
Control	0.25	0
Control	1.17	0
Control	4.3	0.4
Control	6.3	0.9
Control	12.25	4.9
Control	24.0	18.3
A	10.2	0.1
A	25.5	2.6
B	9.2	0.5
B	26.2	18.2
I	8.25	0
I	10.9	0
II	22.6	0
II	27.0	0
III	48.2	0
III	70.5	0.1

A = 0.9% BzOH, comparative

B = 5% EtOH, comparative

I = frozen, thawed at 8.25 hrs. and analyzed, left thawed until 10.9 hrs. for analysis

II = frozen, thawed at 21 hrs., analyzed at 22.6 hrs. and at 27 hrs.

III = frozen, thawed at 48.2 hrs., analyzed, refrozen, then thawed again at 70.5 hrs. for analysis

Samples A and B are not of the invention; samples I, II and III are of the invention. The results are also shown by Figure 1.

Example A (Comparative; Control)

Samples were prepared by the procedure described in Example 1, except that the specific activity of ^{153}Sm was 30, 30, and 50 mCi/mL (Samples C, D, and E, respectively). The results are given in Table A.

These results, along with the control (100 mCi/mL) from Example 1 are shown graphically in Figure 2. This graph demonstrates that the rate of radiolytic degradation is proportional to the specific activity of the radionuclide.

Table A
UNINHIBITED RADIOLYTIC DEGRADATION

Sample	Time (hours)	% Degradation
C	0.3	0
C	2.25	0
C	4.47	0
C	6.75	0
C	11.3	0
C	21.25	0.7
C	35.0	1.4
C	53.0	3.1
D	0.17	0
D	2.42	0
D	4.5	0
D	6.6	0
D	10.75	0
D	20.6	0.4
D	33.8	1.2
D	52.83	2.1
E	2.0	0
E	5.25	0
E	7.6	0
E	11.5	0.2
E	25.5	0.7
E	47.0	6.5

Example 2: Method A, no calcium added

Two solutions were prepared as described in Example 1, except that they contained 27 mCi/mL of ^{153}Sm and were frozen in the same manner as Example 1. The samples were stored in a container filled with powdered dry ice. Samples were taken after allowing the solution to thaw, and then the solution was refrozen. The HPLC procedure of Example 1 was used to analyze the samples. No radiometric peaks other than the one corresponding to the desired product were detected after being frozen for a period of 70 hours.

Example 3: Method A

Three mL of 70 mg/mL of EDTMP solution were pipetted into a beaker. The pH of the solution was adjusted to about 10.26 with 50 percent (weight/weight) NaOH. After placing the solution in a serum vial, the water was removed in a vacuum oven. Three such vials were prepared ("EDTMP kits").

Three 6.0 mL EDTMP kits, made by the above procedure, were reconstituted with $HoCl_3$ at 3×10^{-4} M in 0.1N HCl. Each kit was spiked with 150 µL of ^{166}Ho. The pH of the complex solution was measured for each sample and found to be from 7.6 to 7.7. To one sample (Sample IV) was added $CaCl_2$ to achieve a 1:1 molar ratio of Ca:EDTMP. To another sample (Sample V) was added $CaCl_2$ to achieve a 2:1 molar ratio of Ca:EDTMP. The third sample was the control (without $CaCl_2$ added). Sample IV, upon the addition of $CaCl_2$, had a pH of 7.06 which was then adjusted to a pH of 7.5 with 50 percent (weight/weight) NaOH. Sample V, upon the addition of $CaCl_2$, had a pH of 6.6 which was then adjusted to a pH of 7.5 with 50 percent (weight/weight) NaOH. The percent complexation was found to be 99 percent for all three samples.

Three groups of 3 Sprague-Dawley rats were each injected I.V. with 100 µL of the Control, Sample IV and sample V. After two hours the biodistribution was determined and the results are shown in Table 2.

Table 2

% INJECTED DOSE (^{166}Ho) IN VARIOUS TISSUES

Tissue	Control[d]	Sample IV[d] (1:1)	Sample V[d] (2:1)
Skeleton[a]	53	46	43
Liver	0.07	0.05	0.07
Kidney	0.60	0.30	0.30
Spleen	0.01	0.01	0.01
Muscle[b]	0.34	0.34	0.43
Blood[c]	0.08	0.20	0.22

a was derived by multiplying the % in the femur by 25
b was estimated as 43% of body weight
c was estimated at 6.5% of body weight.
d was average of 3 rats

The data indicates that the biodistribution is similar for all three samples.

Example 4: Method D

A Ca containing-kit (Ca-kit) (1:1 mole Ca:EDTMP) was prepared by stirring the following reagents at pH = 1.97:

Table 3

Reagent	g	mmol	mL
water	--	--	30
EDTMP (96%)	2.198	4.84	--
$Ca(OH)_2$ (95%)	0.3766	4.83	--

The pH was then adjusted with 0.1N NaOH. At pH = 1.97 the solution was clear. At pH = 2.8 to 3 the solution is cloudy. At pH = about 5.8 the solution was clear. At pH = about 10.5 the solution was clear.

The preferred method of preparing the Ca-kit was to weigh out the solid $Ca(OH)_2$ and EDTMP into a beaker; add water and stir until clear; adjust the pH above 6.

The pH can then be adjusted to 9.2 and freeze-dried to obtain the desired formulation in kit form. An addition of Sm solution to the kit forms the ^{153}Sm-EDTMP complex which can then be injected.

Example 5: Method D

^{153}Sm-EDTMP formulation was prepared by reconstituting a 3mL Ca-kit from Example 4 (1:1 mole Ca:EDTMP). The kit was reconstituted by using 3 x 10^{-4}M Sm in 0.1N HCl solution spiked with ^{153}Sm and the pH was adjusted to 7.8 (sample VI). A ^{153}Sm-EDTMP formulation (2:1 Ca:EDTMP) was prepared by a modification of the procedure of Example 4. The kit was reconstituted by using 3 x 10^{-4}M Sm in 0.1N HCl solution spiked with ^{153}Sm and the pH was adjusted to 7.2 (sample VII). Also a 6 mL kit as control without Ca was reconstituted with 6.0 mL of 3 x 10^{-4}M Sm in 0.1N HCl solution spiked with ^{153}Sm and adjusting the pH to 7.5. The percentage of Sm (all Sm isotopes) as a complex was determined by cation exchange chromatography to be >99 percent.

Three Sprague-Dawley rats were injected I.V. with 100 µL of ^{153}Sm-EDTMP solution. The rats were killed 2 hours after injection. The amount of activity in several tissues was determined by comparing the counts in the tissue (measured using a NaI gamma counter) to counts in standard 100 µL volumes of the stock solution and Table 4 shows the results:

Table 4

% INJECTED DOSE (^{153}Sm) IN VARIOUS TISSUES

Tissue	Control[d]	Sample VI[d] (1:1)	Sample VII[d] (2:1)
Skeleton[a]	44	37	38
Liver	0.14	0.15	0.20
Kidney	0.36	0.48	0.48
Spleen	0.01	0.01	0.01
Muscle[b]	0.53	0.60	0.96
Blood[c]	0.21	0.37	0.51

a was derived by multiplying the % in the femur by 25
b was estimated as 43% of body weight
c was estimated at 6.5% of body weight.
d was average of 3 rats

Example 6: Method D

A 3 mL Ca-kit, as prepared in Example 4, was reconstituted with 3.0 mL of 3 x 10^{-4}M $SmCl_3$ in 0.1M HCl. Two mL of the solution were checked for pH and found to be between 7.0 and 8.0 by colorpHast™ pH strip. The solution was spiked with 1-2 µL of ^{153}Sm solution as a tracer. In a similar manner a control kit, with Na, (without Ca, prepared as in Example 3) was reconstituted. Also a Ca-kit reconstituted to contain 48.3 mCi/mL of ^{153}Sm was frozen in dry ice. After 21 days the frozen formulation was thawed and used.

Five Sprague-Dawley rats were injected I.V. with 100 µL solution of the control formulation, five Sprague-Dawley rats were injected with 100 µL of the 21 day old freshly thawed Ca-kit formulation, and 4 Sprague-Dawley rats were injected with 100 µL solution of the Ca-kit formulation (with tracer ^{153}Sm).

The rats were killed 2 hours after injection. The amount of activity in several tissues was determined by comparing the counts in the tissue (measured using a NaI gamma counter) to counts in standard 100 µL volumes of the stock solution. The dose in several tissues is shown in Table 5 below.

Table 5

% INJECTED DOSE (^{153}Sm) IN VARIOUS TISSUES

Tissue	Control[d] No Ca	21 Day Old Frozen Ca-kit[d]	Fresh spiked Ca-kit[e]
Skeleton[a]	53	50	50
Liver	0.24	0.21	0.21
Kidney	0.49	0.41	0.41
Spleen	0.01	0.01	0.01
Muscle[b]	1.18	0.92	0.92
Blood[c]	0.16	0.21	0.20

a was derived by multiplying the % in the femur by 25
b was estimated as 43% of body weight
c was estimated at 6.5% of body weight.
d average of 5 rats
e average of 4 rats

Example 7: Method D

Into a 600 mL beaker containing a magnetic stir bar was added 18.210 g (40.126 mmol) of EDTMP, 2.678 g (36.140 mmol) of $Ca(OH)_2$, and 400 mL of water. The mixture was stirred for 1 hour at room temperature and most of the solids dissolved. The pH was slowly raised using a solution of 50 percent NaOH, and stirring continued until all the solids dissolved. Once the mixture became homogeneous, the pH was slowly raised to 9.2 using a solution of 50 percent NaOH. (The solution turned cloudy at pH 3.6 and became homogenous again at pH 5.8.) The solution was transferred to a 500 mL volumetric flask and was brought to 500 mL by addition of water; the pH was 9.17. The solution was then filtered through a 0.45 micron filter and dispensed into multiple 3, 6 and 18 mL kits. The kits were frozen using a dry ice-acetone bath and then placed into a freeze-dryer. After 4 days the kits were removed, sealed, evacuated, labeled and stored.

A 3 mL Ca-kit from above along with a control (Na) kit, were each reconstituted using 3 mL of $3x10^{-4}$ M Sm in 0.1M HCl spiked with tracer amount of ^{153}Sm (1 to 2 µL). The percent complex was determined by cation exchange chromatography and each was determined to be >99 percent. The pH of both was between 7 and 8. Three 100 µL aliquots from each kit were drawn to be used as standards.

Five Sprague-Dawley rats (150 to 200 g) were injected I.V. with 100 µL of the reconstituted Ca-kit and four Sprague-Dawley rats were injected with 100 µL of the reconstituted control kit. After a period of 2 hours the rats were sacrificed and tissue samples were taken. The tissue samples were counted along with the standards and the biodistribution was determined. The results are shown in Table 6.

Table 6
% INJECTED DOSE (^{153}Sm) IN VARIOUS TISSUES

Tissue	Ca-kit % Dose in[a]	Control Na Kit % Dose in[b]
Bone	50	53
Liver	0.21	0.24
Kidney	0.41	0.49
Spleen	0.01	0.01
Muscle	0.92	1.18
Blood	0.02	0.16
Urine[c]	32	51

a average of 5 rats
b average of 4 rats
c found in paper in cage

The two formulations appear to give equivalent biodistributions.

Example 8 and Comparative Example B: Method D

When a Ca-kit formulation was compared to a Na-kit formulation, with both formulations administered to Sprague-Dawley rats by rapid I.V. injection, the Ca-kit had an LD_{50} of 3.5 times greater than for the Na-kit. Both kits were prepared by the procedure of Example 5. The biodistribution results of the kits were similar. Because of the increased LD_{50}, the Ca-kit provides an added safety factor.

Example 9: Method D

A ^{153}Sm-EDTMP formulation was prepared by reconstituting a 3 mL kit prepared by the procedure of Example 3. The kit was reconstituted by adding 3 mL of ^{153}Sm solution in 0.1M HCl. The reconstituted kit contained 3×10^{-4} M of Sm and 35 mg/mL of EDTMP.

The percentage of Sm as a complex was determined by cation exchange chromatography to be >99 percent. Two Sprague-Dawley rats were injected I.M. in the right thigh and two more rats were injected S.C. over the clavia at the neck. Each rat received 100 µL of ^{153}Sm-EDTMP solution.

The rats were killed 2 hours after injection. The amount of activity in several tissues was determined by comparing the counts in the tissue (measured using a NaI gamma counter) to counts in standard 100 µL volumes of the stock solution. The dose in several tissues is shown in Table 7 below and compared to I.V. injections done similarly.

Table 7
% INJECTED DOSE (^{153}Sm) IN VARIOUS TISSUES

Tissue	S.C.[d]	I.M.[d]	I.V.[d]
Skeleton[a]	49	45	50
Liver	0.14	0.35	0.14
Kidney	0.32	0.32	0.36
Spleen	0.006	0.005	0.001
Muscle[b]	0.40	0.053	0.22
Blood[c]	0.45	0.35	0.12

a was derived by multiplying the % in the femur by 25
b was estimated as 43% of body weight
c was estimated at 6.5% of body weight.
d average of 2 rats

The results from Table 7 indicate that the biodistribution is similar regardless of the mode of administration.

Example 10

Five 3 mL EDTMP kits, prepared by the procedure of Example 4, were reconstituted with the solutions shown in the Table 8 and the pH measured.

Table 8
ACID CONCENTRATIONS

[Ca] M	[HCl] M	pH
0.0375	0.087	7.42±0.03
0.060	0.072	7.40±0.06
0.070	0.065	7.39±0.05

Solutions containing about 1 mCi/mL of ^{153}Sm and a total Sm concentration of 3×10^{-4}M with the Ca content shown in the above Table for each sample and the complex yield determined to be >99 percent for each sample.

The Ca containing acid solutions described in Table 8 were used to titrate existing 3.0 mL EDTMP kits to study the effect of the volume of solution used for reconstitution on final pH. Two mL of each solution was added to each kit and the pH measured. Another 2.0 mL was added in 200 µL aliquots with pH measurements taken after each addition. The results showed that for all of the solutions up to 3.6 mL can be added to a 3.0 mL kit without falling below pH 7.0.

The effect of the added Ca on osmolality of the reconstituted kits was determined. The solutions from Table 8 were used to reconstitute 3.0 mL kits of EDTMP. Following reconstitution, the pH of the solution was measured and the osmolality determined by freezing point depression. The results are shown in Table 9.

Table 9
EFFECT OF ADDED CALCIUM ON SOLUTION MOLALITY

[Ca]	[HCl]	pH	Molality	% of Isotonic
0	0.11	7.50	0.491	164
0.0375	0.087	7.40	0.558	186
0.060	0.072	7.35	0.595	198
0.070	0.065	7.35	0.606	202

The data form Table 9 demonstrates that the kits as formulated are hypertonic and that adding Ca to the radionuclide solutions used to reconstitute to kits only slightly increases the hypertonic result. In a discussion of the influence of formulation on the route of administration of drugs it is known that with intravenous solutions isotonicity becomes less important as long as administration is slow enough to permit dilution or adjustment in the blood [see, for example, P. P. DeLuca and J. C. Boylan, "Formulation of Small Volume Parenterals in Pharmaceutical Dosage Forms", Parenteral Medications Vol. 1, pg. 140, eds. K. E. Avis, L. Lachman, and H. A. Lieberman, pub. Marcel Dekker Inc., N.Y. (1984)].

Example 11: Method D

To test the acute effects of ^{153}Sm-Na-EDTMP and ^{153}Sm-(Ca/Na)-EDTMP upon heart rate and pulse and serum calcium levels shortly after I.V. injection to Beagle dogs the following experiment was preformed. The effects of the rate of infusion were also measured.

The ^{153}Sm-Na-EDTMP, Sample VIII, was prepared from 630 mg of EDTMP and 414 mg NaOH that was lyophilized and sterilized and then reconstituted with sterile Sm, 3 x 10^{-4}M in 0.1M HCl.

The ^{153}Sm-(Ca/Na)-EDTMP, Sample IX, was prepared from 630 mg of EDTMP, 245 mg NaOH and 95 mg $Ca(OH)_2$ that was lyophilized and sterilized and then reconstituted with sterile Sm, 3 x 10^{-4}M in 0.01M HCl.

Each complex used for injection was prepared by adding 18.0 mL of the Sm solution to respective lyophilized EDTMP formulation. The final concentration for the formulation was 35 mg/mL of the Sm-EDTMP complex. The formulations were used within 15 minutes of preparing with the remaining solution frozen for analysis. Analysis of the formulations used for injection confirmed that the injections were at the targeted concentrations.

Young adult male Beagle dogs, approximately 33 weeks of age and weighing from 8.1 to 10.9 kg were used. The dogs were given a complete physical examination by a veterinarian, allowed to acclimate to the laboratory environment for least 30 days, revaccinated against distemper, adenovirus type 2 hepatitis, parainfluenza and parvovirus using Adenoimmune™-7-L (made by Tech America, Biologics Corp.). The dogs were individually identified by a unique number tattooed in the ear by the supplier. The dogs were maintained under the procedures established by the American Association for the Accreditation of Laboratory Animal Care. The laboratory used for the test is accredited.

A single fasted male dog, selected at random, was given a I.V. dose of 30 mg/Kg body weight of either Sample VIII of IX via a catheter inserted into the cephalic vein. The injection site was shaved and prepared with antiseptic solution prior to catheterization. The injection was given at the rate of about 2.0 mL/min (70 mg/min) such that the injection took about four minutes.

Two additional male Beagle dogs were given the same dose level of either Sample VIII or IX as rapidly as possible, about 15 mL/min. The methods of infusions were as before. This was done about 2 weeks after the first injection to verify the previously reported results regarding rate of infusion.

Blood samples were obtained from the jugular vein for analysis of serum calcium and total protein immediately prior to injection and following completion of injection and also at about 5, 15, 30 and 45 minutes and 1, 2 and 4 hrs post-injection. The ventral neck was shaved and prepared with antiseptic solution as above. The dog was monitored for clinical effect during this time period and heart rate and pulse were recorded at each of these time points.

As only individual dogs comprised each of these treatment regimens, summary tables seems inappropriate. However, the slow I.V. injection at a rate of 2.0 mL/min (70 mg/min of the Sm - EDTMP complex) at a dose of 30 mg/kg of body weight of either Sample VIII or IX did not produce any clinical signs. The heart rate increased 7 to 10 percent for each of the formulations; however, this result was considered merely to represent a response to excitement as canine heart rate can vary markedly is response to environmental stimuli. Although there were minimal changes in serum total calcium, the changes are difficult to ascribe to treatment.

When the rate of injection was increased to as fast as was practicable (about 15 mL/min), clinical signs were noted with each of the Samples. Involuntary muscular movements, primarily fine tremors of all muscles, with increased respiration and whining were noted for the dog receiving Sample VIII. These effects were first noted about 20 second after injection began and lasted about 2 min. The dog appeared normal by about 5 min post-injection. The heart rate increased by >50 percent during this same time period. Serum total calcium levels were decreased during this time period; however, this decrease was relatively minor and was accompanied by similar changes in serum total protein. It was impossible to ascribe any definitive effect on total calcium due to injection.

Although the dog receiving Sample IX also showed clinical signs, it is uncertain as to whether these were involuntary movement, as the movements were characteristic of resistance to restraint, as appeared to be the case for the dog that received Sample VIII. The period of increased activity by the dog lasted only about 30 seconds and the dog appeared normal by about 100 seconds post-injection. The heart rate was only increased about 13 percent. Serum calcium level were increased during the post-injection period, but concomitant changed in total protein make this result difficult to interpret.

In summary, dogs receiving 30 mg/kg of either Sample VIII or IX by slow I.V. injection had no effects attributable to the injection. When the injection was given by rapid bolus injection, clinical signs were noted for each sample. The effects were more pronounced with Sample VIII than Sample IX.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Claims

1. A radiopharmaceutical formulation comprising a complex of Samarium-153, Holmium-166, Ytterbium-175, Lutetium-177, Yttrium-90, or Gadolinium-159 with ethylenediaminetetramethylenephosphonic acid or a physiologically-acceptable salt thereof, characterized in that the formulation is frozen, and prior to use is thawed.

2. A radiopharmaceutical formulation of Claim 1, wherein the radionuclide is Samarium-153 or Holmium-166.

3. A radiopharmaceutical formulation of Claim 2, wherein the radionuclide is Samarium-153.

4. A process for preparing a radiopharmaceutical formulation as claimed in any one of Claims 2 or 3, which comprises complexing the Samarium-153 or Holmium-166 with the ethylenediaminetetramethylenephosphonic acid, or a physiologically-acceptable salt thereof, and freezing the complex.

5. A radiopharmaceutical formulation as claimed in any one of Claims 1 to 3, which also comprises a divalent metal.

6. A radiopharmaceutical formulation comprising a complex of a radionuclide with a ligand, or a physiologically-acceptable salt thereof, characterized in that the formulation also comprises a divalent metal.

7. A radiopharmaceutical formulation as claimed in Claim 6, wherein the divalent metal is Fe^{+2}, Mn^{+2}, or an alkaline earth metal ion.

8. A radiopharmaceutical formulation as claimed in Claim 7, wherein the alkaline earth metal ion is Be^{+2} Mg^{+2}, Ca^{+2}, Sr^{+2}, or Ba^{+2}.

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As only individual dogs comprised each of these treatment regimens, summary tables seems inappropriate. However, the slow I.V. injection at a rate of 2.0 mL/min (70 mg/min of the Sm - EDTMP complex) at a dose of 30 mg/kg of body weight of either Sample VIII or IX did not produce any clinical signs. The heart rate increased 7 to 10 percent for each of the formulations; however, this result was considered merely to represent a response to excitement as canine heart rate can vary markedly is response to environmental stimuli. Although there were minimal changes in serum total calcium, the changes are difficult to ascribe to treatment.

When the rate of injection was increased to as fast as was practicable (about 15 mL/min), clinical signs were noted with each of the Samples. Involuntary muscular movements, primarily fine tremors of all muscles, with increased respiration and whining were noted for the dog receiving Sample VIII. These effects were first noted about 20 second after injection began and lasted about 2 min. The dog appeared normal by about 5 min post-injection. The heart rate increased by >50 percent during this same time period. Serum total calcium levels were decreased during this time period; however, this decrease was relatively minor and was accompanied by similar changes in serum total protein. It was impossible to ascribe any definitive effect on total calcium due to injection.

Although the dog receiving Sample IX also showed clinical signs, it is uncertain as to whether these were involuntary movement, as the movements were characteristic of resistance to restraint, as appeared to be the case for the dog that received Sample VIII. The period of increased activity by the dog lasted only about 30 seconds and the dog appeared normal by about 100 seconds post-injection. The heart rate was only increased about 13 percent. Serum calcium level were increased during the post-injection period, but concomitant changed in total protein make this result difficult to interpret.

In summary, dogs receiving 30 mg/kg of either Sample VIII or IX by slow I.V. injection had no effects attributable to the injection. When the injection was given by rapid bolus injection, clinical signs were noted for each sample. The effects were more pronounced with Sample VIII than Sample IX.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

Claims

1. A radiopharmaceutical formulation comprising a complex of Samarium-153, Holmium-166, Ytterbium-175, Lutetium-177, Yttrium-90, or Gadolinium-159 with ethylenediaminetetramethylenephosphonic acid or a physiologically-acceptable salt thereof, characterized in that the formulation is frozen, and prior to use is thawed.

2. A radiopharmaceutical formulation of Claim 1, wherein the radionuclide is Samarium-153 or Holmium-166.

3. A radiopharmaceutical formulation of Claim 2, wherein the radionuclide is Samarium-153.

4. A process for preparing a radiopharmaceutical formulation as claimed in any one of Claims 2 or 3, which comprises complexing the Samarium-153 or Holmium-166 with the ethylenediaminetetramethylenephosphonic acid, or a physiologically-acceptable salt thereof, and freezing the complex.

5. A radiopharmaceutical formulation as claimed in any one of Claims 1 to 3, which also comprises a divalent metal.

6. A radiopharmaceutical formulation comprising a complex of a radionuclide with a ligand, or a physiologically-acceptable salt thereof, characterized in that the formulation also comprises a divalent metal.

7. A radiopharmaceutical formulation as claimed in Claim 6, wherein the divalent metal is Fe^{+2}, Mn^{+2}, or an alkaline earth metal ion.

8. A radiopharmaceutical formulation as claimed in Claim 7, wherein the alkaline earth metal ion is Be^{+2} Mg^{+2}, Ca^{+2}, Sr^{+2}, or Ba^{+2}.

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9. A radiopharmaceutical formulation as claimed in Claim 8, wherein the alkaline earth ion is Ca^{+2}.

10. A radiopharmaceutical formulation as claimed in any one of Claims 6 to 9, wherein the molar ratio of divalent metal to ligand is from 0.25 to 1.

11. A radiopharmaceutical formulation as claimed in Claim 10, wherein the molar ratio of divalent metal to ligand is from 0.5 to 1.

12. A radiopharmaceutical formulation as claimed in Claim 11, wherein the molar ratio of divalent metal to ligand is from 0.75 to 1.

13. A radiopharmaceutical formulation as claimed in Claim 12, wherein the molar ratio of divalent metal to ligand is from 0.9 to 1.

14. A radiopharmaceutical formulation as claimed in any one of Claims 6 to 13, wherein the ligand is ethylenediaminetetramethylenephosphonic acid.

15. A radiopharmaceutical formulation as claimed in Claim 14, wherein the ligand is ethylenediaminetetramethylenephosphonic acid or a physiologically-acceptable salt thereof, and the molar ratio of Ca^{+2} to ethylenediaminetetramethylenephosphonic acid is from 0.9 to 1.

16. A radiopharmaceutical formulation prepared for administration to an animal, which formulation has been prepared by thawing a frozen formulation as claimed in any one of Claims 1 to 5.

17. A process for preparing a divalent metal-containing radiopharmaceutical formulation as claimed in any one of Claims 6 to 16 which comprises:-
(A) adding a divalent metal to a radioactive metal-ligand complex as claimed in any one of Claims 1 to 3; or
(B) freezing, then thawing a radioactive metal-ligand complex as claimed in any one of Claims 1 to 3, then adding a divalent metal; or
(C) refreezing, then thawing the formulation of Step (B); or
(D) adding a divalent metal to a radioactive metal ion solution, then adding a ligand to form the complex; or
(E) freezing the complex from Step (D), then thawing prior to use; or
(F) co-administering a radiopharmaceutical formulation with a divalent metal ion solution using two separate injections at about the same time; or
(G) adding a divalent metal, as its chloride or hydroxide, to a ligand, and then adjusting the pH with a base; or
(H) freeze-drying the solution of Step (G) to form a divalent metal-ligand lyophilized formulation, reconstituting the formulation using an acidic solution containing the radioactive metal ion; or
(I) freezing the complex from Step (H), then thawing prior to use.

18. The process as claimed in Claim 17, wherein the ligand is ethylenediaminetetramethylenephosphonic acid.

19. The process as claimed in Claim 17 or Claim 18, wherein process (H) is carried out, and the acidic solution is a concentration of hydrochloric acid from 0.001 to 1N.

20. The process of Claim 19, wherein the acidic solution is hydrochloric acid having a concentration of from 0.01 to 0.1N.

21. The process as claimed in Claim 4 or in any one of Claims 17 to 20, wherein the freezing was done using dry ice; and optionally acetone, or using liquid nitrogen.

22. The use of a complex of a radionuclide with a ligand, or a physiologically-acceptable salt thereof, together with a divalent metal, in the preparation of a radiopharmaceutical formulation.

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Patentansprüche

1. Radiopharmazeutische Formulierung, welche einen Komplex von Samarium-153, Holmium-166, Ytterbium-175, Lutetium-177, Yttrium-90 oder Gadolinium-159 mit Ethylendiamintetramethylenphosphonsäure oder einem physiologisch verträglichen Salz davon umfaßt, dadurch gekennzeichnet, daß die Formulierung gefroren ist und vor Verwendung aufgetaut wird.

2. Radiopharmazeutische Formulierung nach Anspruch 1, worin das Radionuclid Samarium-153 oder Holmium-166 ist.

3. Radiopharmazeutische Formulierung nach Anspruch 2, worin das Radionuclid Samarium-153 ist.

4. Verfahren zur Herstellung einer radiopharmazeutischen Formulierung nach einem der Ansprüche 2 oder 3, welches Komplexieren des Samarium-153 oder Holmium-166 mit der Ethylendiamintetramethylenphosphonsäure oder einem physiologisch verträglichen Salz davon und Einfrieren des Komplexes umfaßt.

5. Radiopharmazeutische Formulierung nach einem der Ansprüche 1 bis 3, welche auch ein bivalentes Metall umfaßt.

6. Radiopharmazeutische Formulierung, welche einen Komplex eines Radionuclids mit einem Liganden oder einem physiologisch verträglichen Salz davon umfaßt, dadurch gekennzeichnet, daß die Formulierung auch ein bivalentes Metall umfaßt.

7. Radiopharmazeutische Formulierung nach Anspruch 6, worin das bivalente Metall Fe^{2+}, Mn^{2+} oder ein Erdalkalimetallion ist.

8. Radiopharmazeutische Formulierung nach Anspruch 7, worin das Erdalkalimetallion Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+} oder Ba^{2+} ist.

9. Radiopharmazeutische Formulierung nach Anspruch 8, worin das Erdalkalimetallion Ca^{2+} ist.

10. Radiopharmazeutische Formulierung nach einem der Ansprüche 6 bis 9, worin das Molverhältnis des bivalenten Metalls zum Liganden 0,25 bis 1 beträgt.

11. Radiopharmazeutische Formulierung nach Anspruch 10, worin das Molverhältnis des bivalenten Metalls zum Liganden 0,5 bis 1 beträgt.

12. Radiopharmazeutische Formulierung nach Anspruch 11, worin das Molverhältnis des bivalenten Metalls zum Liganden 0,75 bis 1 beträgt.

13. Radiopharmazeutische Formulierung nach Anspruch 12, worin das Molverhältnis des bivalenten Metalls zum Liganden 0,9 bis 1 beträgt.

14. Radiopharmazeutische Formulierung nach einem der Ansprüche 6 bis 13, worin der Ligand Ethylendiamintetramethylenphosphonsäure ist.

15. Radiopharmazeutische Formulierung nach Anspruch 14, worin der Ligand Ethylendiamintetramethylenphosphonsäure oder ein physiologisch verträgliches Salz davon ist und das Molverhältnis von Ca^{2+} zu Ethylendiamintetramethylenphosphonsäure 0,9 bis 1 beträgt.

16. Zur Verabreichung an ein Tier hergestellte radiopharmazeutische Formulierung, welche durch Auftauen einer gefrorenen Formulierung nach einem der Ansprüche 1 bis 5 hergestellt wurde.

17. Verfahren zur Herstellung einer bivalentes Metall enthaltenden radiopharmazeutischen Formulierung nach einem der Ansprüche 6 bis 16, welches umfaßt:
    (A) Zugeben eines bivalenten Metalls zu einem radioaktiven Metall-Ligand-Komplex nach einem der Ansprüche 1 bis 3; oder

(B) Einfrieren eines radioaktiven Metall-Ligand-Komplex nach einem der Ansprüche 1 bis 3, dann Auftauen und dann Zugeben eines bivalenten Metalls; oder
(C) wieder Einfrieren der Formulierung aus Schritt (B) und dann Auftauen; oder
(D) Zugeben eines bivalenten Metalls zu einer Lösung eines radioaktiven Metallions und dann Zugeben eines Liganden unter Bildung des Komplexes; oder
(E) Einfrieren des Komplexes aus Schritt (D) und dann Auftauen vor Verwendung; oder
(F) Verabreichen einer radiopharmazeutischen Formulierung zusammen mit einer Lösung eines bivalenten Metallions unter Verwendung zweier getrennter Injektionen zu etwa der gleichen Zeit; oder
(G) Zugeben eines bivalenten Metalls als dessen Chlorid oder Hydroxid zu einem Liganden und dann Einstellen des pH-Wertes mit einer Base; oder
(H) Gefriertrocknen der Lösung aus Schritt (G), wobei eine lyophilisierte, bivalentes Metall-Ligand-Formulierung gebildet wird, Wiederherstellen der Formulierung unter Verwendung einer sauren Lösung, die das radioaytive Metallion enthält; oder
(I) Einfrieren des Komplexes aus Schritt (H) und dann Auftauen vor Verwendung.

18. Verfahren nach Anspruch 17, wobei der Ligand Ethylendiamintetramethylenphosphonsäureist.

19. Verfahren nach Anspruch 17 oder Anspruch 18, wobei das Verfahren (H) durchgeführt wird und die saure Lösung eine Chlorwasserstoffsäure-Konzentration von 0,001 bis 1 N aufweist.

20. Verfahren nach Anspruch 19, wobei die saure Lösung Chlorwasserstoffsäure mit einer Konzentration von 0,01 bis 0,1 N ist.

21. Verfahren nach Anspruch 4 oder nach einem der Ansprüche 17 bis 20, wobei das Einfrieren unter Verwendung von Trockeneis und gegebenenfalls Aceton oder unter Verwendung von flüssigem Stickstoff erfolgte.

22. Verwendung eines Komplexes eines Radionuclids mit einem Liganden oder einem physiologisch verträglichen Salz davon zusammen mit einem bivalenten Metall bei der Herstellung einer radiopharmazeutische Formulierung.

Revendications

1. Composition radiopharmaceutique, comprenant un complexe du Samarium-153, de l'Holmium-166, de l'Ytterbium-175, du Lutetium-177, de l'Yttrium-90 ou du Gadolinium-159, avec l'acide éthylènediaminetétraméthylènephosphonique ou un sel physiologiquement acceptable de cet acide, caractérisée en ce que la composition est congelée, et est dégelée avant utilisation.

2. Composition radiopharmaceutique selon la revendication 1, dans laquelle le radionucléide est le Samarium-153 ou l'Holmium-166.

3. Composition radiopharmaceutique selon la revendication 2, dans laquelle le radionucléide est le Samarium-153.

4. Procédé de préparation d'une composition radiopharmaceutique telle que revendiquée dans l'une quelconque des revendications 2 et 3, qui comprend la complexation du Samarium-153 ou de l'Holmium-166 avec l'acide éthylènediaminetétraméthylènephosphonique, ou un sel physiologiquement acceptable de cet acide, et la congélation du complexe.

5. Composition radiopharmaceutique selon l'une quelconque des revendications 1 à 3, qui renferme également un métal divalent.

6. Composition radiopharmaceutique comprenant un complexe d'un radionucléide avec un ligand ou un sel physiologiquement acceptable de ce dernier, caractérisée en ce que la composition renferme également un métal divalent.

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7. Composition radiopharmaceutique selon la revendication 6, dans laquelle le métal divalent est Fe^{+2}, Mn^{+2} ou un ion de métal alcalino-terreux.

8. Composition radiopharmaceutique selon la revendication 7, dans laquelle l'ion de métal alcalino-terreux est Be^{+2}, Mg^{+2}, Ca^{+2}, Sr^{+2} ou Ba^{+2}.

9. Composition radiopharmaceutique selon la revendication 8, dans laquelle l'ion de métal alcalino-terreux est Ca^{+2}.

10. Composition radiopharmaceutique selon l'une quelconque des revendications 6 à 9, dans laquelle le rapport molaire du métal divalent au ligand est compris dans l'intervalle allant de 0,25 à 1.

11. Composition radiopharmaceutique selon la revendication 10, dans laquelle le rapport molaire du métal divalent au ligand est compris dans l'intervalle allant de 0,5 à 1.

12. Composition radiopharmaceutique selon la revendication 11, dans laquelle le rapport molaire du métal divalent au ligand est compris dans l'intervalle allant de 0,75 à 1.

13. Composition radiopharmaceutique selon la revendication 12, dans laquelle le rapport molaire du métal divalent au ligand est compris dans l'intervalle allant de 0,9 à 1.

14. Composition radiopharmaceutique selon l'une quelconque des revendications 6 à 13, dans laquelle le ligand est l'acide éthylènediaminetétraméthylènephosphonique.

15. Composition radiopharmaceutique selon la revendication 14, dans laquelle le ligand est l'acide éthylènediaminetétraméthylènephosphonique ou un sel physiologiquement acceptable de cet acide, et le rapport molaire de Ca^{+2} à l'acide éthylènediaminetétraméthylènephosphonique est compris dans l'intervalle allant de 0,9 à 1.

16. Composition radiopharmaceutique préparée en vue d'être administrée à un animal, que l'on a préparée en dégelant une composition congelée, telle que revendiquée dans l'une quelconque des revendications 1 à 5.

17. Procédé de préparation d'une composition radio-pharmaceutique renfermant un métal divalent, telle que revendiquée dans l'une quelconque des revendications 6 à 16, qui comprend :
(A) l'addition d'un métal divalent à un complexe métal radioactif-ligand, tel que revendiqué dans l'une quelconque des revendications 1 à 3; ou
(B) la congélation, puis la décongélation d'un complexe métal radioactif-ligand, tel que revendiqué dans l'une quelconque des revendications 1 à 3, et ensuite l'addition d'un métal divalent; ou
(C) la recongélation, puis la décongélation de la composition de l'étape (B); ou
(D) l'addition d'un métal divalent à une solution d'ions de métal radioactif, puis l'addition d'un ligand pour former le complexe; ou
(E) la congélation du complexe provenant de l'étape (D), puis sa décongélation avant utilisation; ou
(F) la coadministration d'une composition radiopharmaceutique et d'une solution d'ions de métal divalent, au moyen de deux injections séparées, effectuées à peu près au même moment; ou
(G) l'addition d'un métal divalent, sous la forme de son chlorure ou de son hydroxyde, à un ligand, puis le réglage du pH avec une base; ou
(H) la lyophilisation de la solution de l'étape (G) pour former une composition lyophilisée métal divalent-ligand, et la reconstitution de la composition au moyen d'une solution acide contenant l'ion de métal radioactif; ou
(I) la congélation du complexe provenant de l'étape (H), puis sa décongélation avant utilisation.

18. Procédé selon la revendication 17, dans lequel le ligand est l'acide éthylènediaminetétraméthylènephosphonique.

19. Procédé selon la revendication 17 ou 18, dans lequel on réalise l'étape (H) et la solution acide est une solution d'acide chlorhydrique ayant une concentration 0,001N à 1N.

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20. Procédé selon la revendication 19, dans lequel la solution acide est une solution d'acide chlorhydrique ayant une concentration 0,01N à 0,1N.

21. Procédé selon l'une quelconque des revendications 4 et 17 à 20, dans lequel on réalise la congélation en utilisant de la carboglace et éventuellement de l'acétone, ou en utilisant de l'azote liquide.

22. Utilisation d'un complexe d'un radionucléide avec un ligand ou un sel physiologiquement acceptable de ce dernier, et en même temps d'un métal divalent, pour la préparation d'une composition radiopharmaceutique.