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(54) Title: METHODS FOR TREATING RADIOACTIVE CONTAMINATION

(57) Abstract: The present disclosure relates to methods for treating radioactive contamination, in particular internal radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said functionalized polymer is a specific statistic chitosan polymer wherein a part of the monomeric units are functionalized with a chelating moiety which complexes said radionuclide.

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METHODS FOR TREATING RADIOACTIVE CONTAMINATION

The present disclosure relates to methods for treating radioactive contamination, in particular internal radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said functionalized polymer is a specific statistic chitosan polymer wherein a part of the monomeric units are functionalized with a chelating moiety which complexes said radionuclide.

BACKGROUND

During the last decades, an increasing number of scenarios may unfortunately lead to large dissemination of radioactive isotopes in populated area including use of nuclear weapons in either terrorism or war context or incident in nuclear facility like in Fukushima or Chernobyl (Rump A. *et al.*, 2018, *Mil. Med. Res.,* **5**, 27). A nuclear detonation can lead to the release of more than 400 radioactive isotopes on which 40 are of critical concern for human life due to their long radiological half-lives and their important concentrations (Vergara V. B. *et al.,* 2021, *Nutrients,* **13**, *2545*) in organs at risk emphasizing the interest of developing a decorporating agent that can display an efficiency against a large panel of isotopes.

Among these isotopes, 4 are of particular concerns including cesium-137 ($t_{1/2}$ = 30.17 y), cobalt-60 ($t_{1/2}$ = 5.27 y), strontium-90 ($t_{1/2}$ = 28.9 y) and uranium-238 ($4.47.10^9$ y) [2]. Brambilla *et al.* also recently reviewed a list of radionuclides that are more susceptible to be found in a dirty bomb and that represent a large panel (Brambilla S. *et al.*, 2023, *J. Environ. Radioact.,* **263**, *107166*).

During a nuclear event, internal contamination can occur by different ways including ingestion, inhalation, absorption by the skin or due to an open wound (Bodin L. *et al.*, 2021, *J. Radiol. Prot.,* **41**, S427-S437).

Unfortunately, to date only three compounds (only one preventive) have been accepted by FDA and they are limited to the treatment of iodine (by KI in 1978 for preventive protection of the thyroid), of cesium (by prussian blue in 2003) and plutonium and americium (by Zn-DTPA or Ca-DTPA in 2004). Unfortunately, DTPA displays poor solubility and bioavailability and can only be intravenously (iv) administered or aerosolized and in addition has relatively low chelation constants for U(VI) (log K = 16) in comparison to many other endogenous cations (Li Y. *et al.,* 2023, *J. Inorg. Biochem.,* **238**, 112034; Wang X. *et al.*, 2019, *Nature Com.,* **10**, *2570*). In addition, its rapid elimination after iv administration and toxicity concerns avoid its use in a preventative way. Iv administration limits its use in case of

massive treatment of a population in comparison with an oral formulation (Cassatt D. R. *et al.,* 2008, *Radiat. Res.* **170**, *540-548*). In case of contamination, different studies have shown the importance to start the treatment as soon as possible (urgent approach) (Rump A. *et al.,* 2021, *Mil. Med. Res.,* **8**, *3*) requiring easy way of administration and it can be even better to propose preventative drug in case of protection of population soon contaminated or soldiers and or workers going to a contaminated area.

Recently, new per-os biopolymer (Mex-CD1) has been proposed for the treatment of contamination by heavy metals (lead and cadmium) in food (Howard J. A. et al., 2023, Sci. Rep., 13, 2215). The polymer is based on chitosan backbone grafted with DOTAGA (Natuzzi M. et al., 2021, Sci. Rep., 11, 11948).

WO2019/122790 further discloses a medical device which can be inserted into the body for the maintenance of metal homeostasis for therapeutic purposes comprising a chelating moiety for extracting metals.

WO2022/023677 describes a statistical polysaccharide with a weight-average molecular weight of between 100 kDa and 1000 kDa and its use in a dialysis process in order to capture at least one metal, in an magnetic resonance imaging (MRI) process, in a brachytherapy process or in a process for marking foodstuffs to prevent forgeries.

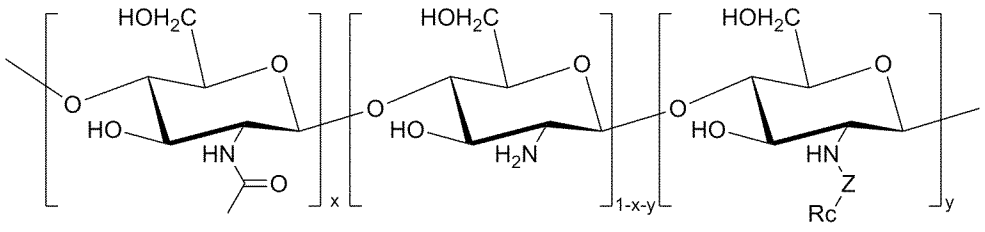
There is therefore still a need to design a safe and efficient treatment for preventing radioactive contamination, in particular internal contamination. More specifically, to the knowledge of the inventors, there is no known oral drug for a preventive treatment for a subject at risk of to be exposed to a radiocontaminant, which would prevent absorption of said radiocontaminant and therefore reduce toxicity associated to internal contamination. There is also no known oral drug for a preventive treatment for a subject at risk of to be exposed to a radiocontaminant, which would further reduce toxicity associated to external contamination through its ROS scavenging ability.

The inventors have now shown that certain specific polymers, when administered orally to mice are at least capable of reducing toxicity associated to radionuclide oral exposure, in particular to Uranium radioisotope internal contamination. More generally, such specific polymers are useful for treating or preventing a subject from internal and/or external contamination.

SUMMARY OF THE INVENTION

The present disclosure relates to A functionalized polymer, for use in a method for treating radioactive contamination, said method comprising orally administering, in a subject at risk

or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said functionalized polymer is a functionalized statistic chitosan of weight average molecular mass between 100kDa and 1000kDa and of formula (I):



(I)

wherein

each Rc is a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

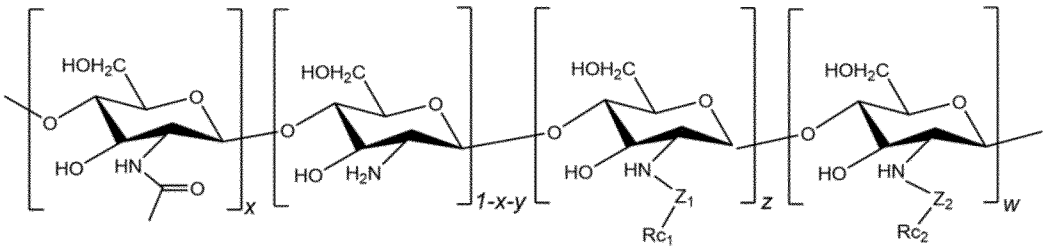
x is between 0.005 and 0.7, preferably between 0.05 and 0.7,

y is between 0.005 and 0.5, preferably between 0.01 and 0.2,

the ratio y/x being greater than or equal to 0.01, preferably greater than or equal to 0.02, and

the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30.

In specific embodiments, said functionalized polymer is a functionalized statistic chitosan of formula (II):



(II)

wherein

Rc_1 is a chelating moiety and Rc_2 is a different chelating moiety or ROS scavenger moiety ,

Z_1 and Z_2, identical or different, are linkers which are a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

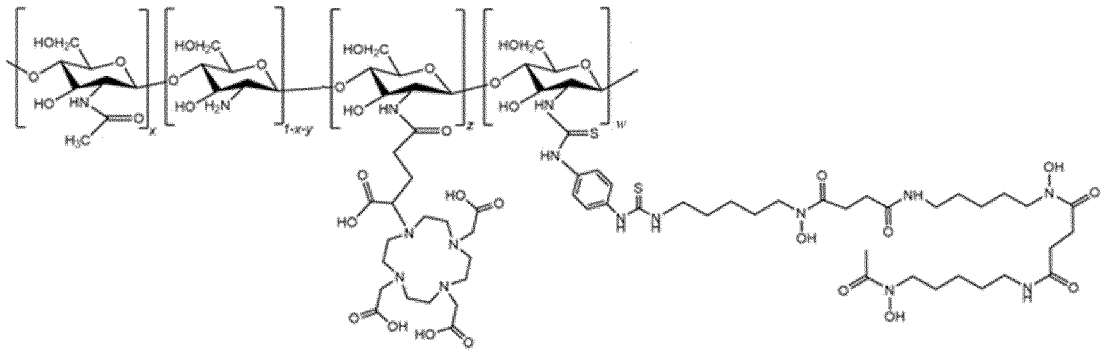
x is between 0.005 and 0.7, preferably between 0.05 and 0.7, and more preferably between 0.2 and 0.6,

y=z+w is between 0.01 and 0.7, preferably between 0.05 and 0.3,

the ratio y/x being greater than or equal to 0.05, preferably greater than or equal to 0.15,

the sum x + y being greater than or equal to 0.15, preferably greater than or equal 0.30, more preferably greater than or equal to 0.35.

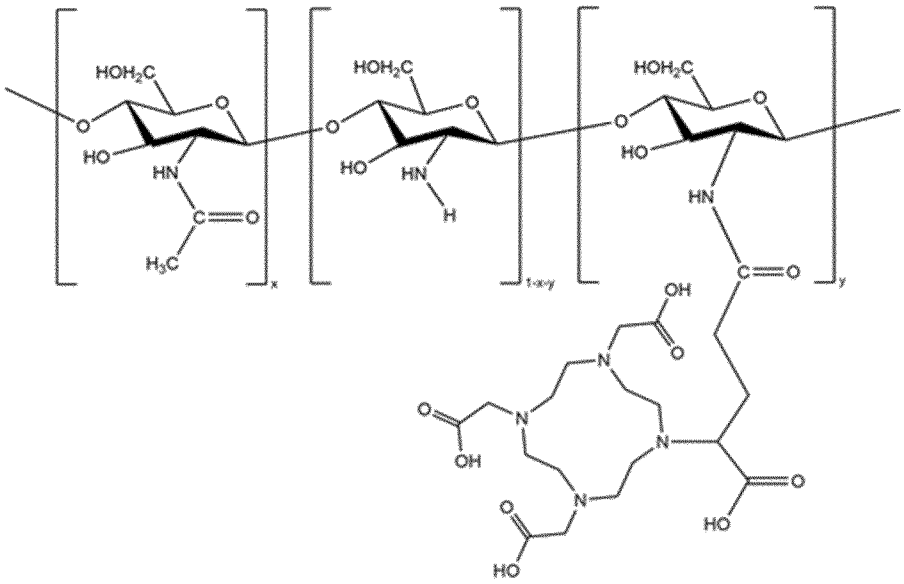
In specific embodiments, the functionalized statistic chitosan has the following formula (IV):



(IV),

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

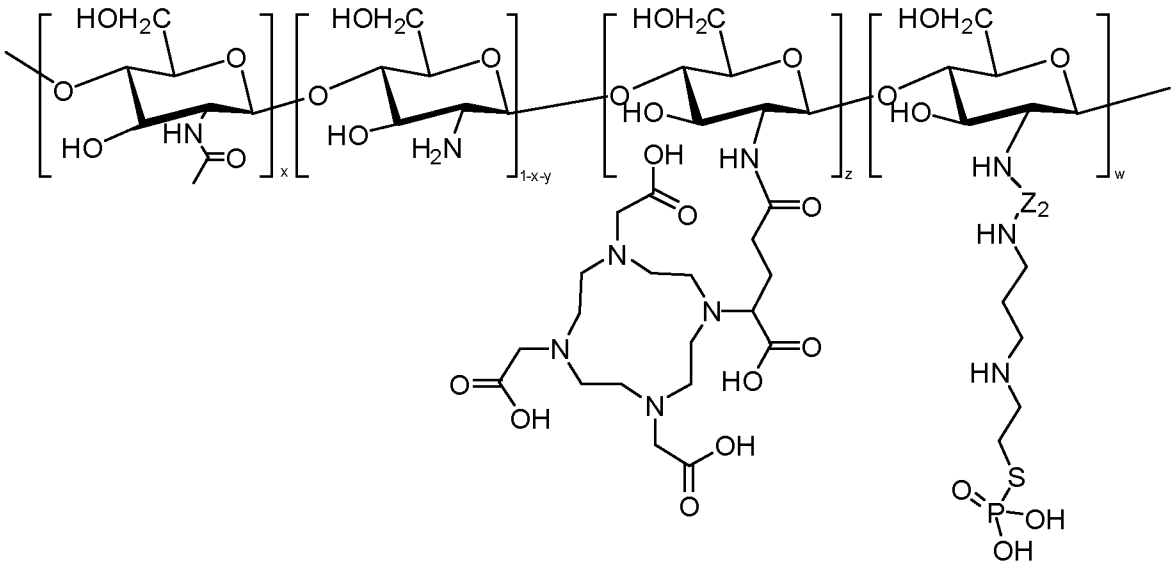
Preferred examples of such functionalized polymer for use according to the present disclosure includes, without limitation, a functionalized polymer of the following formula (III):



(III)

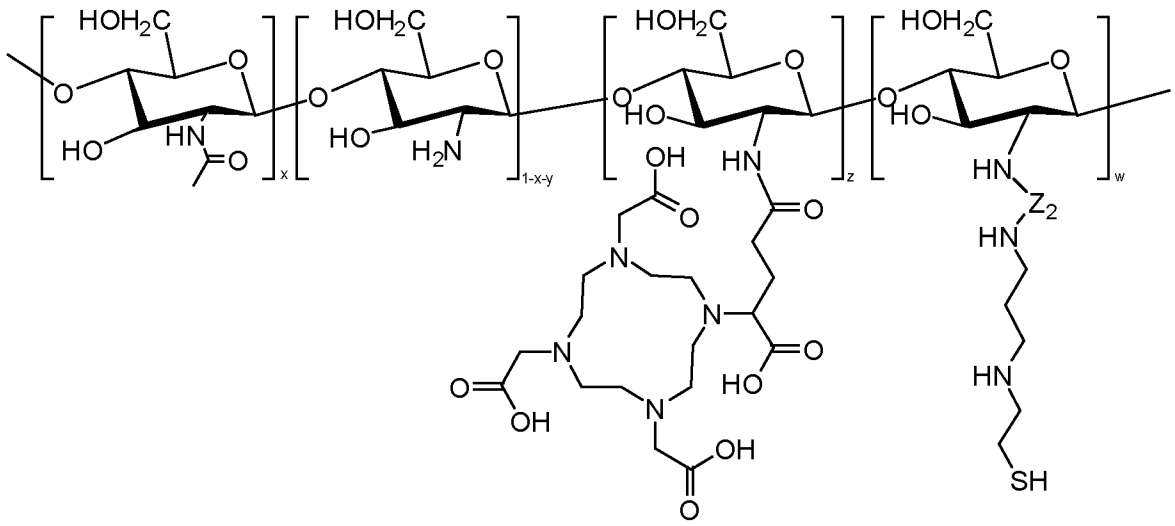
wherein x is between 0.25 and 0.4, typically about 0.3, and y is between 0.05 and 0.2, typically about 0.07,

or of formula (V) or (VI):



(V)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12,



(VI)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

FIGURES

Figure 1: Average Percent of Administered Gd Within Digestive Tract Over 24 h (example 1).

Figure 2: Evolution of mice bodyweight for all 4 groups within the 8 days of the study (example 2.1)

Figure 3: Mice body weight changes at the 4th day of the study just before Uranium administration (left panel) and terminal (right panel) (example 2.1). *p<0.05 compared to the respective group.

Figure 4: Uranium blood and tissue content at the 4th day after Uranium oral exposure (50 mg/kg) in mice given by MexCD1 (50 mg/kg) daily for 7 days starting 3 days before Uranium exposure (example 2.1). Thresholds of detection: blood – 0.03 mg/L, kidney – 0.05 ug/g, spleen – 0.15 ug/g; *p<0.05, **p<0.01, ***p<0.001

Figure 5: Mice all white blood cell (WBC) and their separate subpopulations (lymphocytes – LYM, monocytes and eosinophils – MID, and granulocytes – GRAN) counts, platelet count

(PLT) and their average volume (MPV) as an indicator of platelet matureness (example 5.1). *p<0.05, **p<0.01 compared to the respective group

Figure 6: Mice terminal serum creatinine level (example 2.1). *p<0.05 compared to the respective group.

Figure 7: Estimated percentage of metal amount captured by MEX-CD1 (%chelated = $(m_{OG}-m_{UN})/m_{OG}$) (example 2.3).

Figure 8: Evolution of death index (=PI/confluency) within caco2 cells after administration of different doses of MEX-CD1 (respectively 0 g/L, 0.5 g/L, 1 g/L, 2 g/L, 5 g/L).

Figure 9 : Evolution of death index (=PI/confluency) within caco2 cells after administration of either no treatment, either 2 g/L MEX-CD1, either 1 mM H_2O_2 or both 2 g/L MEX-CD1 and 1 mM H_2O_2.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

In the following, terms as used herein are defined in their meaning.

The term “about” or “ca.” has herein the meaning that the following value may vary for ± 20%, preferably ± 10%, more preferably ± 5%, even more preferably ± 2%, even more preferably ± 1%.

Unless otherwise defined, “%” has herein the meaning of weight percent (wt%), also referred to as weight by weight percent (w/w%).

As used herein, unless specified otherwise, the term "treating" or "treatment", denotes reversing, alleviating, inhibiting the progress of, or preventing, diminishing a disorder or condition to which such term applies, or reversing, alleviating, inhibiting the progress of, or preventing one or more symptoms of the disorder or condition to which such term applies. More preferably, in the context of the present invention, “treating” may include reducing toxicity associated to an exposure to a radiocontaminant, for example by inhalation or ingestion, wound contamination or percutaneous absorption. In specific embodiments, the term “preventing” means at least significantly reducing the toxicity associated to an exposure of a radiocontaminant, wherein the treatment is first orally administered prior to exposure to said radiocontaminant.

As used herein in the context of the present methods of the disclosure, the term “radiocontaminant” also referred as “radioactive material” or “radioactive contaminant”

designate the atoms and cations of radionuclides which are toxic to humans. Radioactive material may be disseminated across an area for example after an accident of an industrial nuclear reactor, or use of a radiological dispersal device (also called “dirty bomb”) or nuclear weapon in a combat zone.

Examples of such radiocontaminants may be Cesium-137, Cesium-134, Strontium-89, Strontium-90, Yttrium-90, Ruthenium-103, Ruthenium-106, Cerium-106, Zirconium-95, Baryum-140, Lanthane-140, Cobalt-60, Cobalt-58, Iron-55, Manganese-54, Zinc-65, Silver-110m, Americium-241, Plutonium-239, Plutonium-238, Plutonium-239, Plutonium-240, Uranium-234, Uranium-235, Uranium-238, Radium-226, Californium-252, Curium-244 and Thorium-232, in particular in the context of a nuclear plant accident.(https://www.irsn.fr/savoir-comprendre/crise/principaux-radionucleides-rejetes-cas-daccident-affectant-centrale#.Y_XaVyaZPEY)

Examples of such radiocontaminants may be Americium-241, Cesium-137, Strontium-90, Yttrium-90, Plutonium-238, Plutonium-239, Plutonium-240, Plutonium-241 in particular in the context of a contamination through a nuclear weapon (McClellan, R. O. Health Effects of Nuclear Weapons and Releases of Radioactive Materials. In *Handbook of Toxicology of Chemical Warfare Agents*; Elsevier, 2020; pp 707–743. https://doi.org/10.1016/B978-0-12-819090-6.00043-X.)

Examples of such radiocontaminants may be Thallium-201, Strontium-90, Cobalt-60, Cesium-137, Iridium-192, Radium-226, Americium-241 in the context of a contamination through a radiological dispersal device (or “dirty bomb”) (Brambilla, S.; Nelson, M. A.; Brown, M. J. 2023, *Journal of Environmental Radioactivity* **2023**, *263*, 107166)

As used herein, the term “internal contamination” refers to the accumulation of a radiocontaminant in targeted organs in the body of a human subject. Internal contamination may arise when said radiocontaminant is ingested, inhaled, or absorbed through the skin or from a contaminated wound.

As used herein, the term “external contamination” refers to toxicity occurring when the radiocontaminant, in the form of dust, powder, or liquid, comes into contact with a person’s skin, hair, or clothing, the contact with the radiocontaminant is external to a person’s body. External contamination generates radical oxygen species in the body which may result in DNA damages and associated disorders, such as cancer. Toxicity associated to external contamination may be treated by radical oxygen species (ROS) scavengers.

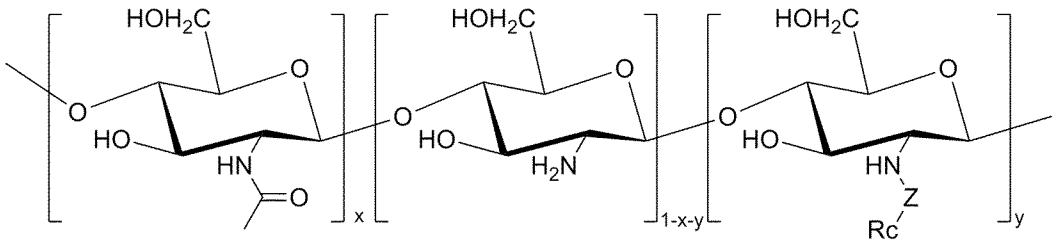
As used herein, the term « ROS scavenger » refers to compounds capable of reacting with reactive oxygen species and other reactive free radicals. This compounds are often thiol-containing molecules, polyphenols or vitamins.. Examples of ROS scavenger compounds include without limitation amifostine, WR-1065, lipoic acid, genistein, apigenin, N-acetylcysteine, cysteine, cysteamine, vitamin A, beta-carotene, vitamin C, recilisib sodium.

As used herein, the terms “effective amount” or “therapeutically efficient amount” of a compound refer to an amount of the compound that will elicit the biological or medical response of a subject, for example, ameliorate the symptoms, alleviate conditions, slow or delay disease progression, or prevent symptoms. In specific embodiment, such therapeutically efficient amount is an amount sufficient to prevent toxicity associated to a radiocontaminant, for example toxicity associated to internal body irradiation of a subject after internal contamination with said radiocontaminant.

The terms “patient”, “subject”, “individual”, and the like, are used interchangeably herein, and refer to a human. In some embodiments, the patient, subject or individual in need of treatment includes those who have already been exposed to radiocontaminants or those who are suspected to have been exposed to radiocontaminants. In some embodiments, the patient, subject or individual in need of treatment includes those who are at risk to be exposed to radiocontaminants

The functionalized polymer for use according to the present disclosure

The functionalized polymer for use in the treatment methods according to the present disclosure is a functionalized chitosan having a statistic macromolecular structure of weight average molecular mass between 100 kDa and 1000 kDa, preferably 200 and 500 kDa, and of formula (I):



(I)

wherein

each Rc is independently a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and

optionally contains one or more unsaturations and being able to contain one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,
x is between 0.005 and 0.7, preferably between 0.05 and 0.7,
y is between 0.01 and 0.7, preferably between 0.05 and 0.2,
the ratio y/x being greater than or equal to 0.05, preferably greater than or equal to 0.15, and
the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30, more preferably greater than or equal to 0.35.

As used herein, the term “chelator” or “chelating agent” or “chelating moiety” is used to define a chemical structure or moiety which exhibits a relatively high affinity for certain elements and displays at least two coordination sites. The affinity is such that the chelator is able to chelate the radiocontaminant, i.e., a radionuclide, and more specifically a radionuclide selected from the group consisting of Manganese-54, Iron-55, Cobalt-58, Cobalt-60, Zinc-65, Strontium-89, Strontium-90, Yttrium-90, Zirconium-95, Ruthenium-103, Ruthenium-106, Cerium-106, Silver-110m, Cesium-134, Cesium-137, Baryum-140, Lanthane-140, Iridium-192, Thallium-201, Radium-226, Thorium-232, Uranium-234, Uranium-235, Uranium-238, Plutonium-238, Plutonium-239, Plutonium-240, Plutonium-241, Americium-241, Curium-244, and, Californium-252.

In specific embodiments, the chelating moiety exhibits a high affinity for chelating a radioactive isotope of Uranium, Thorium, Thallium, Cesium, Strontium, Iridium and Cobalt, or mixtures thereof.

A chelator may function primarily to “neutralize” said radiocontaminant by maintaining their chelation to these metals and preventing their reactions and interactions with other biomaterials. A chelator may exhibit a pincer-type structure or moiety with two or more opposed portions formed by chemical groups that have negative charges within biological environments (sulfhydryl groups, ketone groups, carboxy groups, hydroxyl groups, etc.) or neutral charge (amino group). These groups are spaced accordingly to allow for the comfortable accommodation of the metal ion within their structure.

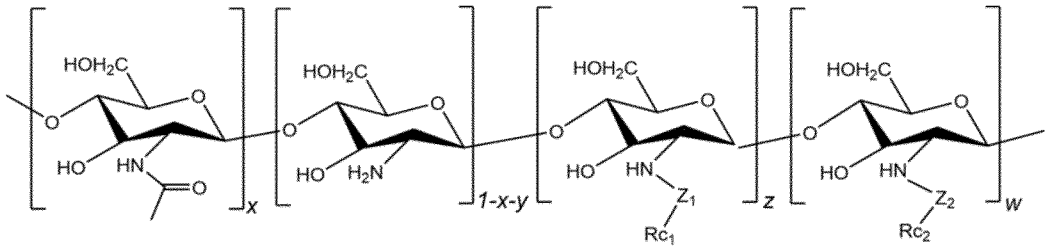
As used herein, the term “independently” for the Rc moiety i.e. the chelating moiety, means that each Rc of the functionalized statistic chitosan of formula (I) is a chelating moiety that may be different from one to another, or may be identical. For example, each Rc is identical i.e., there is one type of Rc throughout the functionalized statistic chitosan, or there may be more than one type of Rc throughout the functionalized statistic chitosan i.e. two, three, four, five or even n different Rc, n being an integer. They are all independently selected

from the groups carrying a chelating moiety. The same applies to the Z-linkers, several Z-linkers may be present, and they may be identical or different from each other.

In an embodiment, in formula I, x is between 0.005 and 0.6; y is between 0.1 and 0.9; the ratio y/x being greater than 0.16 ; and the sum x + y being greater than 0.30.

In a specific embodiment, the functionalized statistic chitosan of the present disclosure has a complexation constant of at least 10^{15} for a d or f transition element.

In an embodiment, the functionalized statistic chitosan of formula I is a functionalized statistic chitosan of formula (II):



(II)

wherein

Rc_1 is a chelating moiety and Rc_2 is a different chelating moiety or a ROS scavenger moiety ,

Z_1 and Z_2, identical or different, are linkers which are a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

x is between 0.005 and 0.7, preferably between 0.05 and 0.7, and more preferably between 0.2 and 0.6,

y=z+w is between 0.01 and 0.7, preferably between 0.05 and 0.3,

the ratio y/x being greater than or equal to 0.05, preferably greater than or equal to 0.15,

the sum x + y being greater than or equal to 0.15, preferably greater than or equal 0.30, more preferably greater than or equal to 0.35. In another embodiment, in formula II, x is between 0.005 and 0.6; y is between 0.1 and 0.9; the ratio y/x being greater than 0.3; and z is between 0.5 and 1.

Preferred Rc moiety for polymers of Formulae I and II (Rc, Rc1 and Rc2)

As used herein, the term “Rc moiety” refers to Rc moiety of formula I, and the terms “Rc1 and Rc2 moiety” refer to Rc_1 and Rc_2 of formula II, when Rc_2 is present. According to the present disclosure, Rc_1 group is a chelating moiety and Rc_2 group is either a chelating moiety or a ROS scavenger moiety. In other words, the Rc and Rc_1 moieties enable chelation of one or more metals by forming a complex; optionally Rc_2 can also chelate one or more metals by forming a complex or is a ROS scavenger moiety.

In specific embodiments, each of the Rc, Rc_1 and, optionally, Rc_2 moiety may comprise two or more coordination sites. Preferably, the coordination site is a nitrogen or oxygen atom. Advantageously, each of the Rc, Rc_1 and, optionally, Rc_2 moiety comprises between 4 and 8 coordination sites, more preferably between 6 and 8 coordination sites and even more preferably each of the Rc, Rc_1 and, optionally, Rc_2 moiety comprises 6 coordination sites.

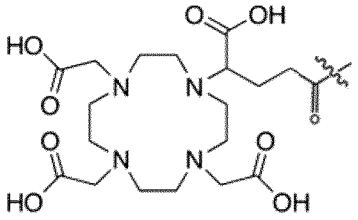
As used herein, the term “coordination site” refers to a single function capable of complexing a radionuclide. For example, an amine function represents a coordination site by the formation of a dative bond between the nitrogen atom and the metal, and a hydroxamic acid function also represents a coordination site by the formation of a dative bond between the oxygen of the carbonyl unit and by a covalent bond with the oxygen of the N-oxide unit the coordination site thus forming a five-membered ring.

In an embodiment, for the functionalized statistic chitosan of formula I, each Rc moiety is independently selected from the group consisting of DOTA (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-teracetic acid), NOTA (1,4,7-triazacyclononane-1,4, 7-triacetic acid), NODAGA (1,4,7-triazacyclononane-1-glutaric-4,7-diacetic acid), DOTAGA (2-(4,7,10-tris(carboxymethyl)-1,4,7, 10-tetraazacyclododecan-1-yl)pentanedioic acid), DOTAM (1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclodecane), NOTAM (1,4,7-tetrakis(carbamoylmethyl)-1, 4,7-triazacyclononane), DOTP (1,4,7,10-tetraazacyclododecane 1,4,7,10-tetrakis(methylene phosphonate), NOTP (1,4,7-tetrakis(methylene phosphonate)-1, 4,7-triazacyclononane), TETA (1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-teracetic acid), TETAM (1,4,8, 11-tetraazacyclotetradecane-N,N',N'',N'''-tetrakis(carbamoyl methyl), DTPA (diethylene triaminopentaacetic acid) Bz-DFO (benzyl deferoxamine) and DFO (deferoxamine), preferably from the group consisting of DOTAGA, Bz- DFO, DFO, DOTAM and DTPA, and more preferably the Rc group is DOTAGA.

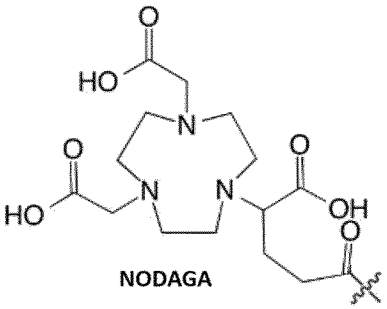
In another embodiment, for the functionalized statistic chitosan of formula II, Rc1 and Rc2 are independently selected from the group consisting of DOTA, NOTA, NODAGA, DOTAGA,

DOTAM, NOTAM, DOTP, NOTP, TETA, TETAM, DTPA, Bz-DFO and DFO, preferably from the group consisting of DOTAGA, Bz-DFO, DFO, DOTAM and DTPA.

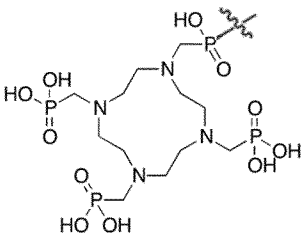
In an embodiment, the chelating moiety is selected from the group consisting of:



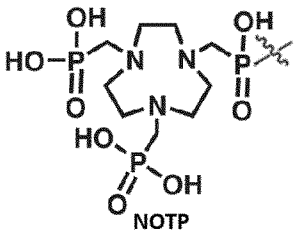
DOTAGA



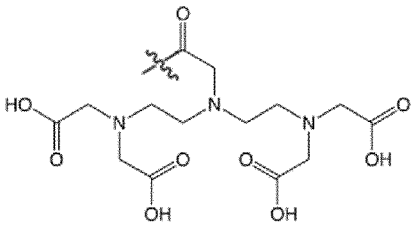
NODAGA



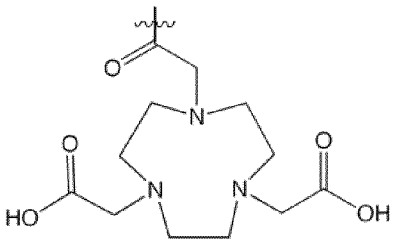
DOTP



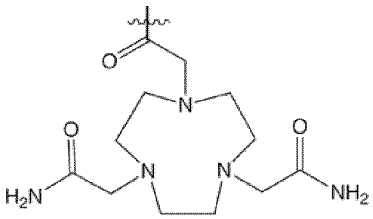
NOTP



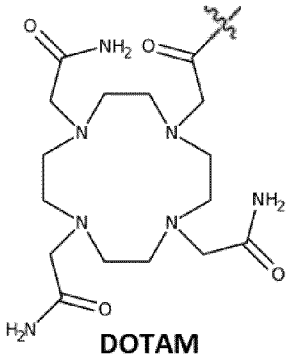
DTPA



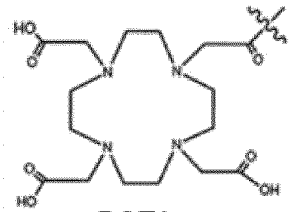
NOTA



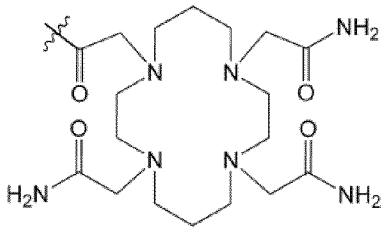
NOTAM



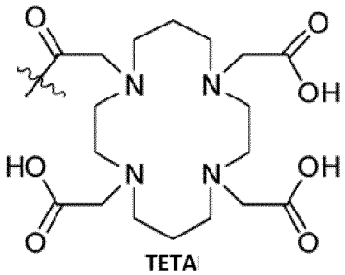
DOTAM



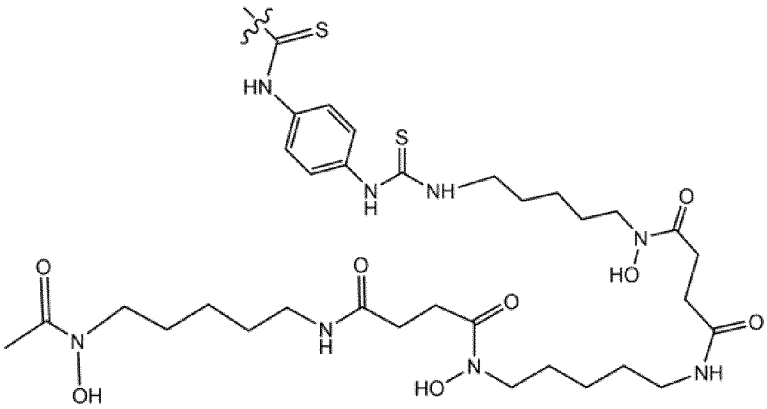
DOTA



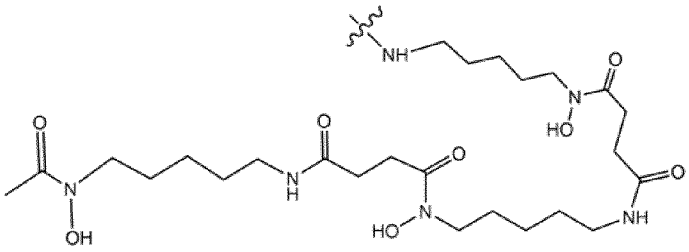
TETAM



TETA



Bz-DFO

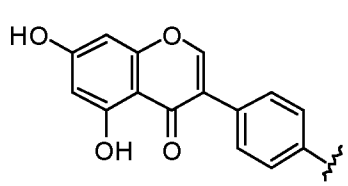
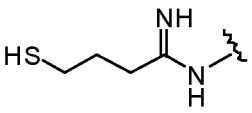
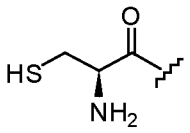
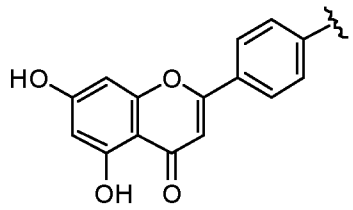
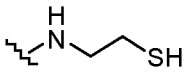
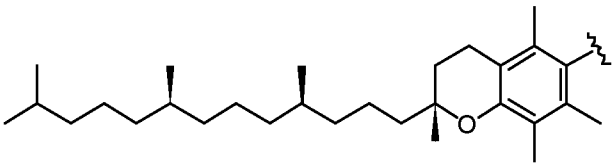
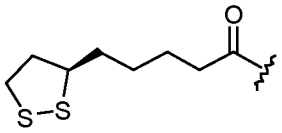
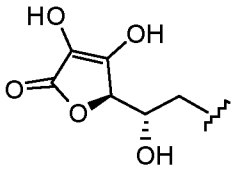
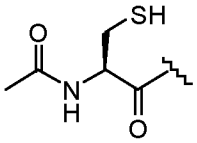
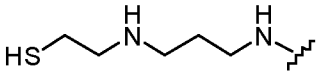
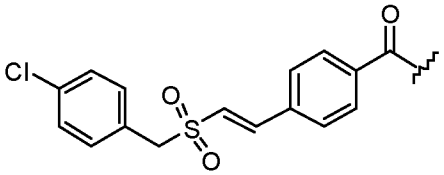
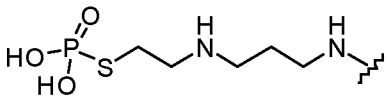


DFO

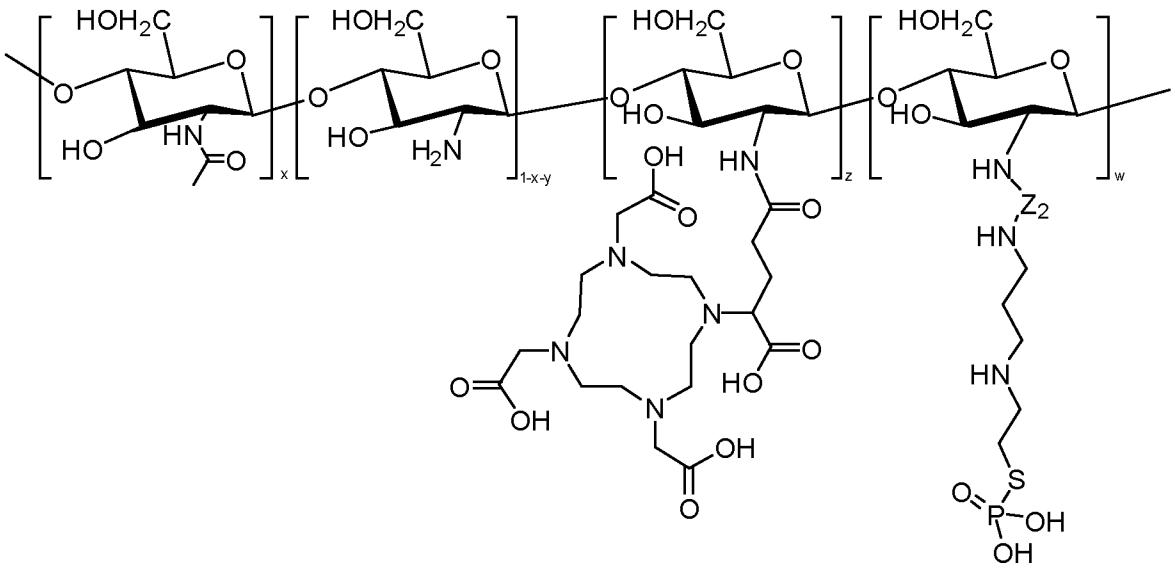
In an embodiment, for the functionalized statistic chitosan of formula I, the group Rc is DOTAGA, and preferably, z/y=1.

In another embodiment, for the functionalized statistic chitosan of formula II, the group Rc1 is DOTAGA and the group Rc2 is Bz-DFO.In an embodiment, for the functionalized statistic chitosan of formula II, Rc2 is a ROS scavenger moiety independently selected from the group consisting of 4-thio-butyl-amidine, amifostine, WR-1065, lipoic acid, genistein, apigenin, N-acetylcysteine, cysteine, cysteamine, vitamin A, beta-carotene, vitamin C, vitamin E, resilisib.

In specific embodiments for the functionalized statistic chitosan of formula II, Rc1 is a chelating moiety, for example DOTA or DOTAGA moiety, and Rc2 is selected from the following ROS scavenger moieties:

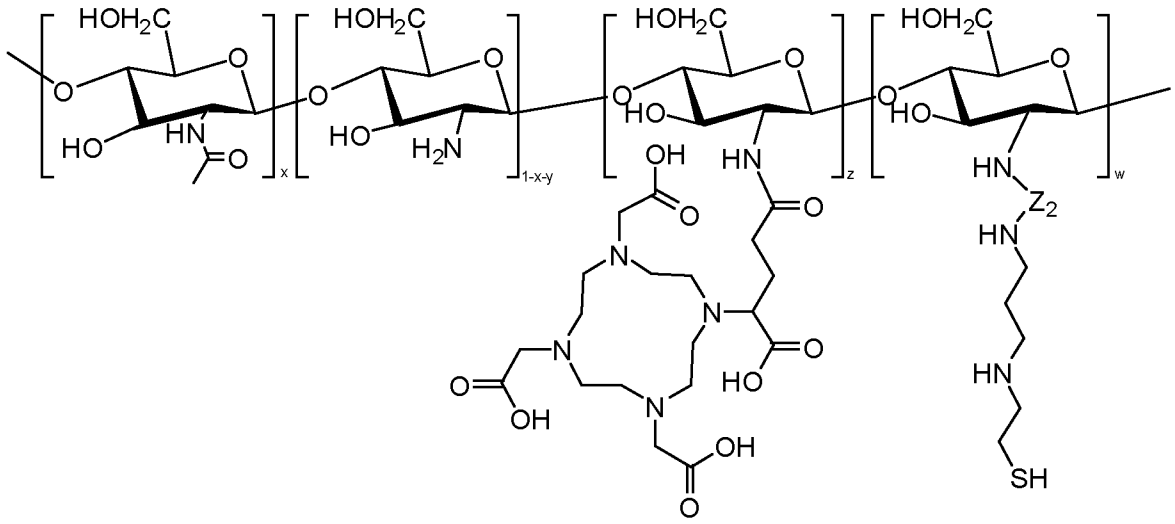
An example of a functionalized statistic chitosan of formula II is according to formula (V):



(V)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

Another example of a functionalized statistic chitosan of formula II is according to formula (VI):



(VI)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

Preferred Z linkers for polymers of formulae I and II (Z, Z1 and Z2)

As used herein, the term "Z linkers" refers to Z linker of formula I, and the terms "Z_1 and Z_2 linkers" refer to Z_1 and Z_2 linkers of formula II, when the Z_2 binder is present.

The choice of the Z, Z_1 and Z_2 linkers in formula I and II depends essentially on the Rc, Rc_1 and Rc_2 moieties and the metal to be chelated. Indeed, for stearic reasons in particular, the Rc, Rc_1 and Rc_2 moieties may be more or less close to the 6-membered ring of the nitrogen of the glucosamine unit.

In formula I, each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturation and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens.

In an embodiment, in formula I, each Z is independently selected from the group consisting of: a bond, a linear or branched alkyl chain having between 1 and 12 carbon atoms, and a linear or branched alkenyl chain having between 2 and 12 carbon atoms, said alkyl and alkenyl chains may be interrupted by one or more C6-C10 aryl groups, and/or by one or more heteroatoms or groups selected from the group consisting of -O-, -S-, -C(O)-, -NR'-, -C(O)NR'-, -NR'-C(O)-, -NR'-C(O)-NR'-, -NR'-C(O)-O-, -O-C(O)NR', -C(S)NR'-, -NR'-C(S)-, -NR'-C(S)-NR, said alkyl and alkenyl chains may be substituted with one or more groups selected from the group consisting of halogen, -OR', -COOR', -SR', -NR'2, each R' being independently H or C1-C6 alkyl.

Advantageously, in Formula I, each Z is independently selected from the group consisting of: a bond and a linear or branched alkyl chain having between 1 and 12 carbon atoms, said alkyl chain may be interrupted by one or more C6-C10 aryl groups, and/or by one or more heteroatoms or groups selected from the group consisting of -O-, -S-, -C(O)-, -NR'-, -C(O)NR'-, -NR'-C(O)-, -C(S)NR'-, -NR'-C(S)-NR', each R' being independently H or C1-C6 alkyl.

In an embodiment each Z is an alkyl chain having between 1 and 12 carbon atoms.

In another embodiment, each Z is a polyethylene glycol (PEG).

Advantageously, in Formula II, Z_1 and Z_2 are independently a single bond or a hydrocarbon chain having between 1 and 12 carbon atoms, wherein said chain may be linear or branched and may have one or more unsaturations and may have one or more heteroatoms, preferably selected from nitrogen, oxygen, sulfur, and halogens.

In an embodiment, in formula II, Z_1 and Z_2 are independently selected from the group consisting of: a bond, a linear or branched alkyl chain having between 1 and 12 carbon atoms, and a linear or branched alkenyl chain having between 2 and 12 carbon atoms, said alkyl and alkenyl chains may be interrupted by one or more C6-C10 aryl groups, and/or by one or more heteroatoms or groups selected from the group consisting of -O-, -S-, -C(O)-, -NR'-, -C(O)NR'-, -NR'-C(O)-, -NR'-C(O)-NR'-, -NR'-C(O)-O-, -O-C(O)NR', -C(S)NR'-, -NR'-C(S)-, -NR'-C(S)-NR, said alkyl and alkenyl chains may be substituted with one or more groups selected from the group consisting of halogen, -OR', -COOR', -SR', -NR'2, each R' being independently H or C1-C6 alkyl.

In an embodiment, in Formula II, Z_1 and Z_2 are independently selected from the group consisting of: a bond and a straight or branched alkyl chain having between 1 and 12 carbon atoms, wherein said alkyl chain may be interrupted by one or more C6-C10 aryl groups, and/or by one or more heteroatoms or groups selected from the group consisting of -O-, -S-, -C(O)-, -NR'-, -C(O)NR'-, -NR'-C(O)-, -C(S)NR'-, -NR'-C(S)-NR', each R' being independently H or C1-C6 alkyl.

In a specific embodiment Z_1 and/or Z_2 is an alkyl chain having between 1 and 12 carbon atoms.

In another specific embodiment, Z_1 and/or Z_2 is a polyethylene glycol (PEG).

Preferred monomeric units arrangement of the functionalized statistic chitosan of formulae I and II

The functionalized statistic chitosan in formulae I for use in the methods of the disclosure is composed of 3 different monomeric units, namely an N-acetyl glucosamine type A unit, a glucosamine type B unit and a glucosamine type C unit functionalized by a chelating moiety (of the Rc type) linked by a linker (of the Z type) to the nitrogen of the glucosamine.

The functionalized statistic chitosan is statistic polymer. In other words, the sequence of the individual monomer units A, B and type C is random.

The functionalized statistic chitosan in formulae II of the present disclosure is composed of 4 different monomeric units, namely an N-acetyl glucosamine type A unit, a glucosamine type B unit and two glucosamine type C unit, namely C1 and C2, functionalized by a chelating moiety (of the Rc1 type or Rc2 type) linked by a linker (of the Z1 type or Z2 type) to the nitrogen of the glucosamine.

The functionalized statistic chitosan in formulae II is statistic polymer. In other words, the sequence of the individual monomer units A, B, C1 and C2 is random.

In formulae I and II, x represents the proportion of A units and x is between 0.005 and 0.7, preferably between 0.05 and 0.7, more preferably between 0.2 and 0.6, even more preferably x is between 0.25 and 0.4, typically about 0.3. In an embodiment, x is between 0.025 and 0.075, more preferably between 0.04 and 0.06, typically about 0.05.

In formulae I and II, y represents the proportion of C-type units and y is between 0.01 and 0.7, preferably between 0.05 and 0.2. In an embodiment, y is between 0.03 and 0.2, preferably between 0.05 and 0.1, even more preferably between 0.07 and 0.08, typically about 0.072. In another embodiment, y is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.15 or 0.12.

In another embodiment, when the Rc1 and Rc2 of formula II are independently selected from the group consisting of DOTA, NOTA, NODAGA, DOTAGA, DOTAM, NOTAM, DOTP, NOTP, TETA, TETAM, DTPA, Bz-DFO and DFO, preferably from the group consisting of DOTAGA, Bz-DFO, DFO, DOTAM and DTPA, and y is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12, Rc1 is between 0.06 and 0.08, typically about 0.07 and Rc2 is between 0.04 and 0.06, typically about 0.05.

The rest of the monomer units in formulae I and II are B units. Thus, in formulae I and II, the proportion of B units is equal to 1-x-y.

According to the disclosure, in formulae I and II, the ratio y/x is greater than or equal to 0.05, preferably greater than or equal to 0.15. Indeed, effectiveness of the functionalized statistic chitosan is determined by the number of chelation sites, which is directly related to the number of metals required, for example, for reducing local inflammation induced by and/or

inducing a deregulation of metal homeostasis and for reducing oxidative stress, in a subject in need thereof.

To be administered as a solution to a subject in need thereof while being effective, the functionalized statistic chitosan must be soluble at physiological pH, i.e. pH of between 4.8 and 8. For this purpose, the sum of x + y may be greater than or equal to 0.15, preferably greater than or equal to 0.30, more preferably greater than or equal to 0.35.

The combination of the particular ratio between the number of A units and the number of C units and the sum of the proportion of A units and the proportion of C units makes it possible to obtain adequate chelation and solubility allowing the functionalized statistic chitosan to be used in the treatment of diseases characterized by local inflammation induced by and/or inducing a deregulation of metal homeostasis and increased oxidative stress, in a subject in need thereof.

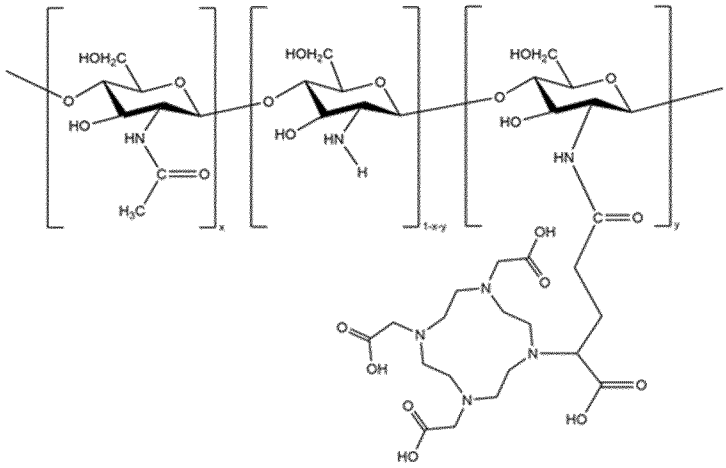
In accordance with the invention, z/y is between 0.5 and 1. In other words, the C-type units may be exclusively units having Z1 as a linker and Rc1 as a chelating moiety-bearing group.

In an embodiment, the functionalized statistic chitosan is selected from the following functionalized statistic chitosan:

- a functionalized statistic chitosan of formula II where z/y = 1, Rc1 is DOTAGA and Z1 is a bond;
- a functionalized statistic chitosan of formula II where z/y = 1, Rc1 is DTPA and Z1 is a bond; and
- a functionalized statistic chitosan of formula II where $0.5 \leq z/y < 1$, Rc1 is DOTAGA and Z1 is a bond, and Rc2 is Bz-DFO and Z2 is selected from the group consisting of: a bond and a straight or branched alkyl chain having between 1 and 12 carbon atoms, wherein said alkyl chain may be interrupted by one or more C6-C10 aryl groups, and/or by one or more heteroatoms or groups selected from the group consisting of -O-, -S-, -C(O)-, -NR'-, -C(O)NR'-, -NR'-C(O)-, -C(S)NR'-, -NR'-C(S)-NR', each R' being independently H or C1-C6 alkyl.

The preferred functionalized polymers of formula (III) or (IV) for use in the present methods of treatment of the disclosure

In a more specific embodiment, the functionalized statistic chitosan has the following formula (III):



(III)

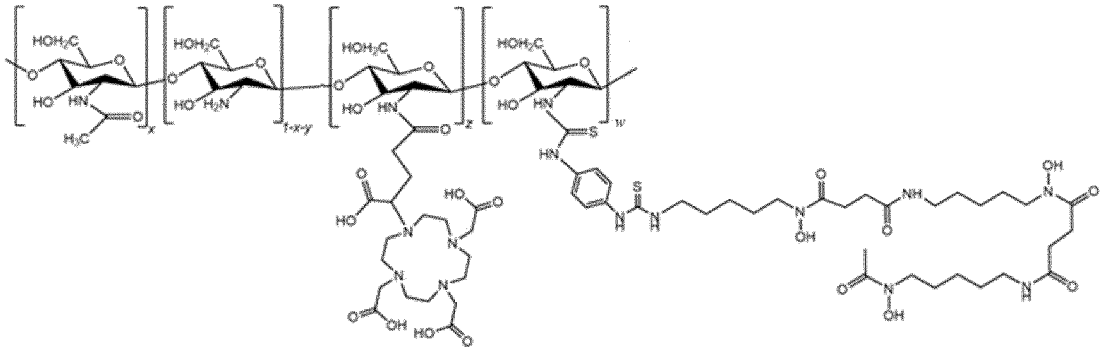
wherein x is between 0.25 and 0.4, typically about 0.3, and y is between 0.05 and 0.2, typically about 0.07.

In another embodiment, the functionalized statistic chitosan has the formula (III), wherein x is between 0.025 and 0.075, more preferably between 0.04 and 0.06, typically about 0.05, and y is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.15.

In certain embodiment, the functionalized polymer has a weight average molecular mass between 100 kDa and 1000 kDa, preferably between 150 kDa and 750 kDa, more preferably between 200 kDa and 500 kDa, even more preferably between 250 kDa and 400 kDa, and even more preferably about 300 kDa.

In a specific embodiment, the functionalized statistic chitosan is soluble in aqueous solution at physiological pH i.e. pH of between 4.8 and 8 and responds the following : $(DS_{DOTAGA}(\%)+3.5)*(DA(\%)+8) \geq 150$ wherein DS is the degree of substitution of the DOTAGA and DA is the degree of acetylation of the functionalized statistic chitosan.

In another embodiment, the functionalized statistic chitosan has the following formula (IV):



(IV),

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

Synthesis of the functionalized polymers of formula I and II

The polymers of formulae I and II (encompassing the polymer of formulae III and IV) can be synthesized using the methods disclosed in WO 2022/023677 and in the reference *Natuzzi, M., Grange, C., Gréa, T. et al. Feasibility study and direct extraction of endogenous free metallic cations combining hemodialysis and chelating polymer. Sci Rep 11, 19948 (2021).*

Pharmaceutical composition of the functionalized polymer

The functionalized polymer for use is administered orally as such or may be formulated in the form with one or more pharmaceutically acceptable excipients.

The term "pharmaceutically acceptable excipients", as used herein, refers to a non-active substance that is added alongside the drug substance, and is part of the formulation mixture. Pharmaceutically acceptable excipients are for example fillers, solvents, diluents, carriers, auxiliaries, distributing and sensing agents, delivery agents, such as preserving agents, disintegrants, moisteners, emulsifiers, suspending agents, thickeners, sweeteners, flavouring agents, aromatizing agents, antibacterial agents, fungicides, lubricants, and prolonged delivery controllers, antioxidants, glidants. The choice and suitable proportions of them are depended on the nature and way of administration and dosage.

Any suitable excipients known to those of ordinary skill in the art for use in pharmaceutical compositions may be employed in the compositions described herein.

In a specific embodiment, the pharmaceutical composition may be a liquid form suitable for oral administration, such as an aqueous solution of said functionalized polymer. In another

specific embodiment, the pharmaceutical composition may be a solid dosage form suitable for oral administration. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In a preferred embodiment, the pharmaceutical composition is a capsule or a tablet.

In an embodiment, the release of the capsule or tablet content may be immediate or modified such as delayed, targeted or extended. In a preferred embodiment the solid dosage form is an immediate release dosage form.

A second object of the disclosure pertains to an oral formulation comprising a functionalized polymer of the disclosure, and one or more pharmaceutically acceptable excipients.

In an embodiment, the pharmaceutically acceptable excipients comprises fillers, disintegrants, lubricants, glidants.

Fillers

Fillers, (also referred to as a diluents, dilutants or thinners) are substance which are added to the drug substance in order to make the latter suitable for oral administration (e.g., capsules, tablets). Fillers themselves should not produce any pharmacological effect on human being. Examples of fillers include mannitol, microcrystalline cellulose, lactose monohydrate, anhydrous lactose, corn starch, xylitol, sorbitol, sucrose, dicalcium phosphate, maltodextrin, and gelatin.

Disintegrants

Disintegrants are added to oral solid dosage forms to aid in their disaggregation. Disintegrants are formulated to cause a rapid break-up of solids dosage forms when they come into contact with moisture. Disintegration is typically viewed as the first step in the dissolution process. Examples of disintegrants include the modified starch such as sodium starch glycolate, sodium carboxymethyl starch, and pre-gelatinized starch, crosslinked polymers, such as crosslinked polyvinylpyrrolidone (crospovidone) or crosslinked sodium carboxymethyl cellulose (croscarmellose sodium), and calcium silicate.

Lubricants

Lubricants are substances that we use in tablet and capsule formulations in order to reduce the friction. Lubricant can facilitate extrusion of tablets from matrix, thus preventing formation of scratches on their surfaces. By nature lubricants can be divided into two groups: a) fats and fat-like substances; b) powdery substance. Powdery substances are more applicable then the fat-like ones, because the latter impact on solubility and chemical

stability of the tablets. Powdery lubricants are introduced by powdering of granulate. They provide constant-rate outflow of mass for tabletizing from hopper into matrix that guaranties accuracy and constancy of the drug substance dosage.

Examples of lubricants include magnesium stearate, hydrogenated castor oil, glyceryl behenate, calcium stearate, zinc stearate, mineral oil, silicone fluid, sodium lauryl sulfate, L-leucine, and sodium stearyl fumarate.

Glidants

Glidants are blended with the formulation to enhance the tablet-core blend-material flow property. During the early stage of compression, glidants are mixed within the particle arrangement of the tablet powder blend to improve flowability and uniformity within the die cavity of tablet presses. Glidants encourage the flow of tablet granulation by diminishing friction between particles. The effect of glidants on the flow of the granules depends on the size and shape of the particles of the granules and the glidants. Above a certain concentration, the glidant will in fact function to inhibit flowability. In tablet manufacture, glidants are usually added just prior to compression. Examples of glidants include colloidal silicon dioxide, starch, magnesium stearate and talc.

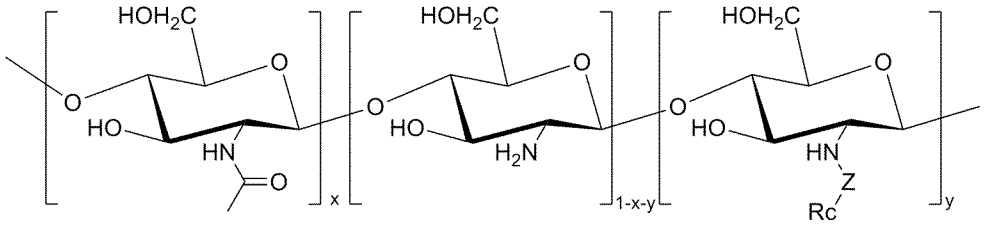
Any suitable excipients known to those of ordinary skill in the art in pharmaceutical compositions may be further employed in the compositions described herein.

In specific embodiments, the unit dose of said functionalized polymer ranges between 0.1 mg and 500 mg, preferably between 1 mg and 100 mg, for example between 2 mg and 10 mg, for example about 5 mg.

<u>The methods of use</u>

The functionalized polymer and their pharmaceutical compositions as described in the previous sections are useful as a drug in methods for treating radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination.

In specific embodiments, the present disclosure relates to a method for treating radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said functionalized polymer is a functionalized statistic chitosan of weight average molecular mass between 100kDa and 1000kDa and of formula (I):



(I)

wherein

each Rc is a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

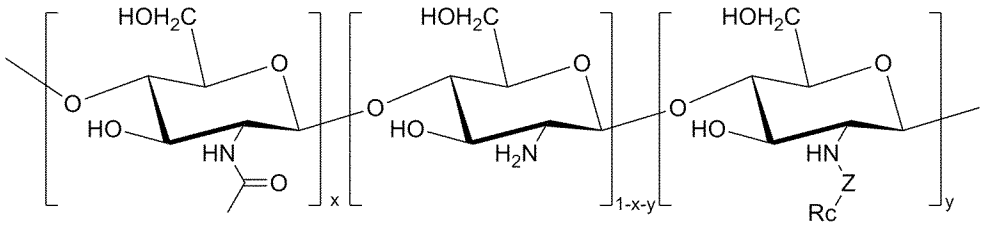
x is between 0.005 and 0.7, preferably between 0.05 and 0.7,

y is between 0.005 and 0.5, preferably between 0.01 and 0.2,

the ratio y/x being greater than or equal to 0.01, preferably greater than or equal to 0.02, and

the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30.

In specific embodiments, the present disclosure relates to the use of a functionalized polymer method for the manufacturing of a medicament for treating radioactive contamination in a subject , said functionalized polymer is a functionalized statistic chitosan of weight average molecular mass between 100kDa and 1000kDa and of formula (I):



(I)

wherein

each Rc is a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or

branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

x is between 0.005 and 0.7, preferably between 0.05 and 0.7,

y is between 0.005 and 0.5, preferably between 0.01 and 0.2,

the ratio y/x being greater than or equal to 0.01, preferably greater than or equal to 0.02, and

the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30.

Any of the functionalized polymers as described in the previous sections, in particular of formula (I), (II), (III), (IV) and (V) may advantageously selected for the methods of treatment disclosed herein.

In specific embodiments, said subject is a mammal, for example a human subject, at risk or suspected of radioactive contamination.

For example, radioactive contamination can arise from accidents involving nuclear reactors, industrial sources, or medical sources. Radioactive contamination may also arise as a result of criminal or terrorist actions. Radioactive contamination may arise from dissemination of radioactive material across an area, typically a combat zone, by a “radiological dispersal device” (“dirty bomb”) using conventional (non-nuclear) explosives.

Subject “at risk” may therefore include soldiers in a combat zone, or rescue workers, or any subject susceptible to be present in an area of potential contamination.

Subject “suspected” may therefore include a subject who has been present in a contaminated area or in an area suspected to be contaminated.

In specific embodiments, a subject suitable for the disclosed treatment methods is a human subject at risk, or suspected, of radioactive contamination with a radionuclide selected from Manganese-54, Iron-55, Cobalt-58, Cobalt-60, Zinc-65, Strontium-89, Strontium-90, Yttrium-90, Zirconium-95, Ruthenium-103, Ruthenium-106, Cerium-106, Silver-110m, Cesium-134, Cesium-137, Baryum-140, Lanthane-140, Iridium-192, Thallium-201, Radium-226, Thorium-232, Uranium-234, Uranium-235, Uranium-238, Plutonium-238, Plutonium-239, Plutonium-240, Plutonium-241, Americium-241, Curium-244, and, Californium-252.

In specific embodiments, a subject suitable for the disclosed treatment methods is a human subject at risk, or suspected, of radioactive contamination with a radionuclide selected from

radioactive isotopes of Uranium, Thorium, Thallium, Cesium, Strontium, Iridium and Cobalt, or mixtures thereof.

In specific embodiments, a subject suitable for the disclosed treatment methods, is a human subject at risk or suspected of internal radioactive contamination. Internal contamination may occur via inhalation of the radioactive contaminant, ingestion, or through contaminated wound. Indeed, without wishing to be bound by any theory, the inventors believe that, by remaining in the gastrointestinal tract, the functionalized polymer as used herein advantageously acts locally by retaining a portion of the ingested radionuclides in the gastrointestinal tract for example by chelation, for example after accidental radioactive contamination (typically from nuclear reactors, industrial sources or medical sources), or criminal or terrorist actions or at a combat zone, prior to their assimilation and therefore, preventing their passage through the intestinal barrier. The fact that the polymer does not pass the intestinal barrier therefore allows a local action in the gastro-intestinal tract and avoids the common adverse effects of the presence of a chelating moiety in the systemic compartment as observed in prior art treatments. In addition, it is known that even by inhalation, part of the radionuclide can pass the gastro-intestinal barrier and can be then captured by the functionalized polymers. Lastly, by limiting passage through the intestinal barrier, it also enables sufficient excretion of any radionuclide which has been internalized, for example through other routes, such as inhalation route and avoid or reduce their accumulation in the targeted organs.

Accordingly, in a specific embodiment, an efficient amount is an amount of said functionalized polymer as orally administered which reduces or prevents gastro-intestinal absorption of said radiocontaminant, and therefore reduces internal contamination by ingestion or inhalation.

In specific embodiments, for example, an efficient amount of said functional polymer for use in the treatment methods reduces the uptake of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% of the radioactive contaminant in a subject exposed to said radioactive contaminant compared to a subject not treated with said functionalized polymer of the disclosure and similarly exposed to said radioactive contaminant.

In specific embodiments, for example, an efficient amount of said functional polymer for use in the treatment methods reduces the uptake of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% of a radionuclide selected from Manganese-54, Iron-55, Cobalt-58, Cobalt-60, Zinc-65, Strontium-89, Strontium-90, Yttrium-90, Zirconium-95, Ruthenium-103, Ruthenium-106, Cerium-106, Silver-110m, Cesium-134, Cesium-137, Baryum-140, Lanthane-140, Iridium-192, Thallium-201, Radium-226, Thorium-232, Uranium-234,

Uranium-235, Uranium-238, Plutonium-238, Plutonium-239, Plutonium-240, Plutonium-241, Americium-241, Curium-244, and, Californium-252, in a subject exposed to said radionuclide, compared to a subject not treated with said functionalized polymer of the disclosure and similarly exposed to said radionuclide.

In specific embodiments, said functionalized polymer reduces the uptake of a radionuclide is selected from the group consisting of Cesium-137, Cesium-134, Strontium-89, Strontium-90, Yttrium-90, Ruthenium-103, Ruthenium-106, Cerium-106, Zirconium-95, Baryum-140, Lanthane-140, Cobalt-60, Cobalt-58, Iron-55, Manganese-54, Zinc-65, Silver-110m, Americium-241, Plutonium-239, Plutonium-238, Plutonium-239, Plutonium-240, Uranium-234, Uranium-235, Uranium-238, Radium-226, Californium-252, Curium-244 and Thorium-232, wherein said subject is exposed to external and/or internal contamination through a nuclear plant accident.(https://www.irsn.fr/savoir-comprendre/crise/principaux-radionucleides-rejetes-cas-daccident-affectant-centrale#.Y_XaVyaZPEY)

In specific embodiments, said functionalized polymer reduces the uptake of a radionuclide selected from the group consisting of Americium-241, Cesium-137, Strontium-90, Yttrium-90, Plutonium-238, Plutonium-239, Plutonium-240, and Plutonium-241, wherein said subject in particular in the context of a contamination through a nuclear weapon.

In specific embodiments, said functionalized polymer reduces the uptake of a radionuclide selected from the group consisting of Thallium-201, Strontium-90, Cobalt-60, Cesium-137, Iridium-192, Radium-226, and Americium-241, wherein said subject is exposed external and/or internal contamination through a radiological dispersal device (or “dirty bomb”).

In more specific embodiments, said functionalized polymer reduces the uptake of Uranium isotope, and said subject is exposed to Uranium isotope radioactivity, for example due to nuclear accident or use of radiological dispersal device (also called “dirty bomb”).

The skilled person will determine the specific dosing regimen, such the appropriate dose, suitable duration and frequency of administration of the functionalized polymer considering different factors, including the type and severity of the internal contamination (or risk of internal contamination), the patient, etc.

Typical daily dose may comprise between 0.1 mg and 500 mg, preferably between 1 mg and 100 mg, more preferably between 2 mg and 10 mg, even more preferably about 5 mg, preferably the functionalized statistic chitosan.

In specific embodiments, the functionalized polymer is first administered at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours prior to first exposure

to said radiocontaminant. Preferably, the functionalized polymer is first administered at least 12 hours prior to first exposure to said radiocontaminant.

In specific embodiments, the functionalized polymer is administered once, twice or thrice a day to the subject in need thereof, for example for at least 3, 4, 5, 6, 7 days following the period of exposure to the radiocontaminant.

The functionalized polymer may be administered in combination with one or more additional drugs, including for example

- free chelating agents for accelerating elimination via excretion by the kidneys, such as Ca-DTPA or Zn-DTPA,
- blocking and diluting agents to reduce uptake to target tissues, such as potassium iodide (KI) for radioactive iodine,
- mobilizing agents, i.e. compounds that enhance and increasing the natural turnover process of radioactive contaminants and accelerating their release from tissues, including propylthiouracil, ammonium chloride, diuretics, expectorants, and inhalants, parathyroid extract, and corticosteroids, or other drugs.

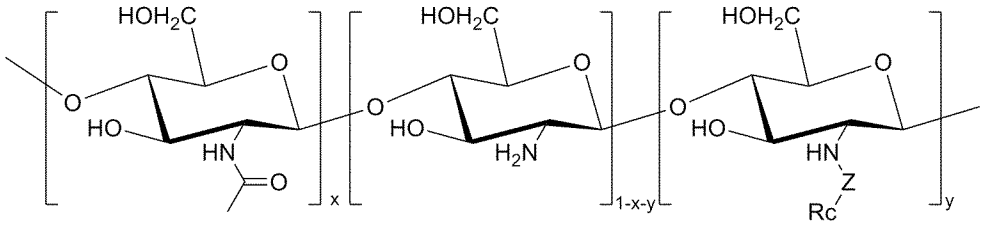
As used herein “combination” refers to either a fixed combination in one dosage unit form, or a combined administration where a compound of the present disclosure and a combination partner (e.g. another drug as explained above,) may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect. The single components may be packaged in a kit or separately. One or both of the components (e.g., powders or liquids) may be reconstituted or diluted to a desired dose prior to administration. The terms “co-administration” or “combined administration” or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (e.g. a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

SPECIFIC EMBODIMENTS

Specific embodiments of the methods of use as disclosed herein are described hereafter:

Embodiment 1: A functionalized polymer, for use in a method for treating radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said

functionalized polymer is a functionalized statistic chitosan of weight average molecular mass between 100kDa and 1000kDa and of formula (I):



(I)

wherein

each Rc is a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

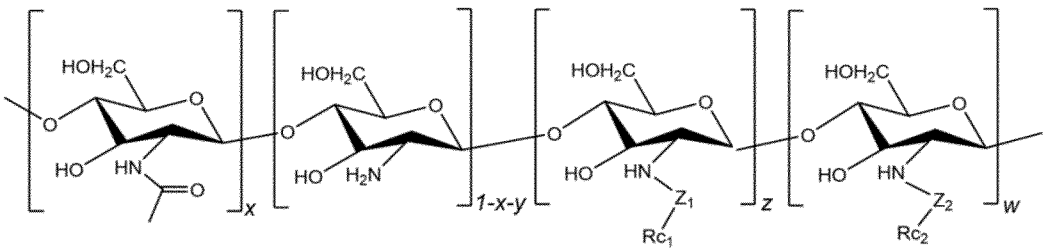
x is between 0.005 and 0.7, preferably between 0.05 and 0.7,

y is between 0.005 and 0.5, preferably between 0.01 and 0.2,

the ratio y/x being greater than or equal to 0.01, preferably greater than or equal to 0.02, and

the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30.

Embodiment 2: A functionalized polymer, for use according to Embodiment 1, wherein said functionalized polymer is a functionalized statistic chitosan of formula (II):



(II)

wherein

Rc_1 is a chelating moiety and Rc_2 is a different chelating moiety or ROS scavenger moiety ,

Z_1 and Z_2, identical or different, are linkers which are a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

x is between 0.005 and 0.7, preferably between 0.05 and 0.7, and more preferably between 0.2 and 0.6,

y=z+w is between 0.01 and 0.7, preferably between 0.05 and 0.3,

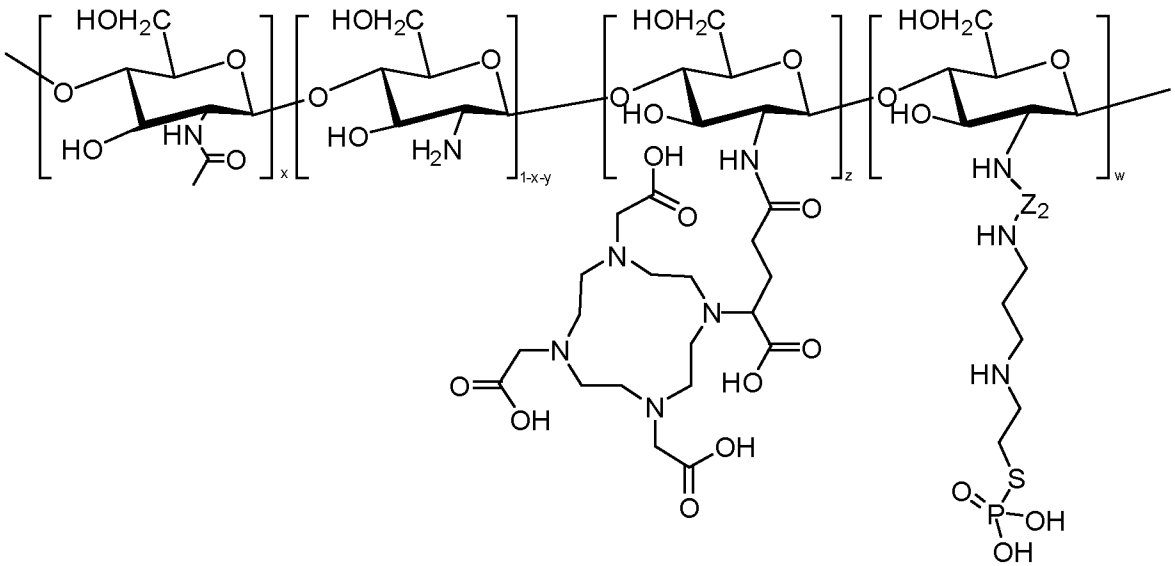
the ratio y/x being greater than or equal to 0.05, preferably greater than or equal to 0.15,

the sum x + y being greater than or equal to 0.15, preferably greater than or equal 0.30, more preferably greater than or equal to 0.35.

Embodiment 3: The functionalized polymer of formula II, for use according to Embodiment 2, wherein x is between 0.005 and 0.6; y is between 0.1 and 0.9; the ratio y/x being greater than 0.3; and z is between 0.5 and 1.

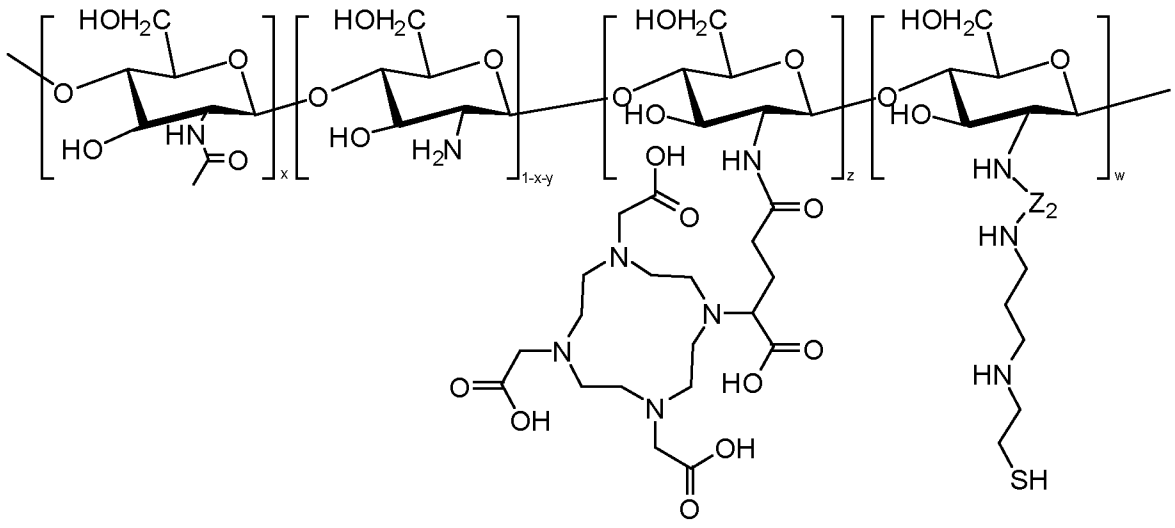
Embodiment 4: The functionalized polymer of formula II, for use according to Embodiment 2 or 3, wherein Rc2 is a ROS scavenger moiety independently selected from the group consisting of 4-thio-butyl-amidine, amifostine, WR-1065, lipoic acid, genistein, apigenin, N-acetylcysteine, cysteine, cysteamine, vitamin A, beta-carotene, vitamin C, vitamin E, resilisib.

Embodiment 5: The functionalized polymer of formula II, for use according to any one of Embodiments 2 to 4, wherein said functionalized polymer is a functionalized statistic chitosan of formula (V) or (VI):



(V)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.



(VI)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

Embodiment 6: The functionalized polymer for use according to any one of Embodiments 1-5, for preventing from internal contamination by a radiocontaminant.

Embodiment 7: The functionalized polymer for use according to any one of Embodiments1-6,, which further acts as a radical oxygen species (ROS) scavenger for reducing toxicity associated to external radioactive contamination.

Embodiment 8: The functionalized polymer for use according to any one of Embodiments 1-7, wherein said radiocontaminant is a radionuclide selected from Americium-241, Californium-252, Cesium-141, Cobalt-60, Uranium-232, Uranium -233, Uranium -234, Uranium -235, Uranium-236, Uranium-238, Plutonium-238, Plutonium-239, Plutonium-240, Plutonium-241, Strontium-89, Strontium-90, Cesium-135, Cesium-137, Cerium-144, Zirconium-93, Zirconium-95, Rutenium-106, Technicium-99, Tin-126, Iridium-192, Polonium-210, Radium-226, Thorium-232, and Thalium-201.

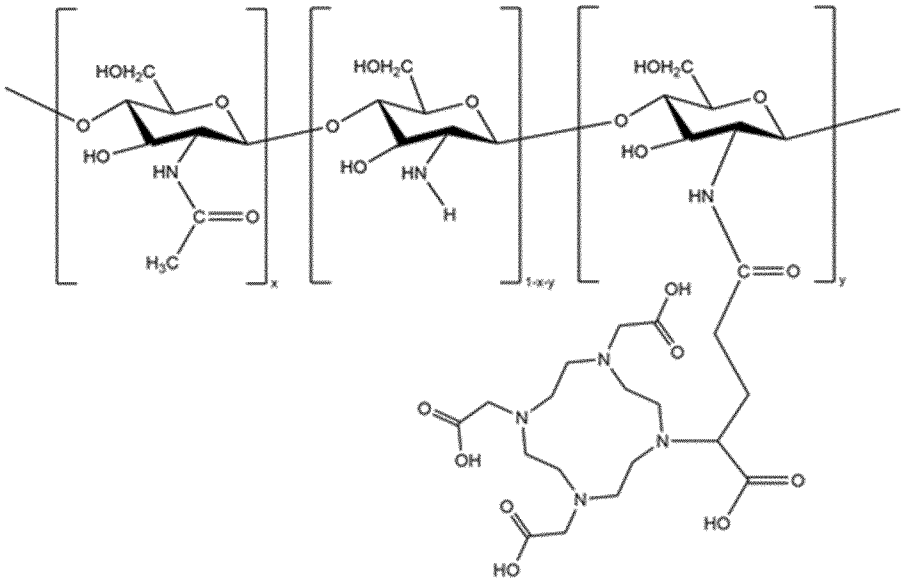
Embodiment 9: The functionalized polymer for use according to Embodiment 8, wherein said radiocontaminant is a radionuclide selected from a radioactive isotope of Uranium, Thorium, Thalium, Cesium, Strontium, Iridium and Cobalt, or mixtures thereof.

Embodiment 10: The functionalized polymer for use according to any one of Embodiments1-9, wherein oral administration of said functionalized polymer reduces or prevents gastro-intestinal absorption of said radiocontaminant.

Embodiment 11: The functionalized polymer for use according to any one of Embodiments 1-10, wherein said chelating moiety is selected from the group consisting of DOTA, NOTA, NODAGA, DOTAGA, DOTAM, NOTAM, DOTP, NOTP, TETA, TETAM, DTPA, Bz-DFO, DFO and mixtures thereof, preferably from the group consisting of DOTAGA, Bz-DFO, DFO, DOTAM, DTPA and mixtures thereof.

Embodiment 12: The functionalized polymer for use according to any one of Embodiments 1 to 11, wherein said functionalized statistic chitosan has a weight average molecular mass between 200 kDa and 500 kDa.

Embodiment 13: The functionalized polymer for use according to any one of Embodiments 1 and 6-12, having the following formula (III):



(III)

wherein x is between 0.25 and 0.4, typically about 0.3, and y is between 0.05 and 0.2, typically about 0.07.

Embodiment 14: The functionalized polymer for use according to any one of Embodiments 1 to 13, wherein the functionalized polymer is administered to the subject at a unit dose of between 0.1 mg and 500 mg, preferably between 1 mg and 100 mg, more preferably between 2 mg and 10 mg, even more preferably about 5 mg.

Embodiment 15: The functionalized polymer for use according any to one of Embodiments 1 to 14, wherein the functionalized polymer is daily administered once, twice or thrice a day.

Embodiment 16: The functionalized polymer for use according any to one of Embodiments 1 to 15, wherein the functionalized polymer is first administered at least 6 hours, preferably at least 12 hours prior to first exposure to said radiocontaminant.

Embodiment 17: The functionalized polymer for use according to any one of Embodiments 1 to 16, wherein said subject will be, is, or has been exposed to radioactive contamination due to accidents involving nuclear reactors.

Embodiment 18: The functionalized polymer for use according to any one of Embodiments 1 to 17, wherein said subject will be, is, or has been exposed to radioactive contamination due to presence of said subject in a zone during or after an accident involving a nuclear reactor.

Embodiment 19: The functionalized polymer for use according to any one of Embodiments 1 to 18, wherein said subject is at risk of exposure to radioactive contamination due to presence in a combat zone during or after the use of a nuclear explosive or a radiological dispersal device.

Hereinafter, the present disclosure is further described in more details and specifically with reference to the examples, which however are not intended to limit the present invention.

EXAMPLES

Synthesis of functionalized polymer of the invention:

MEX-CD1 (Chitosan as polymer and DOTAGA as chelating moiety)

In the first step, 60 g of chitosan, 4 L of ultra-pure water, and 45 mL of glacial acetic acid are introduced into a reactors of 10 L and agitated for 16 hours at a pH of 4.5 ± 0.5. A solution of pale yellow is observed.

In the second step, 1.2 L of 1,2-propanediol is added to the pale yellow solution obtained from step 1 and agitated for 1 hour. A solution composed of 14 mL of acetic anhydride in

600 mL of 1,2-propanediol is slowly added over 10 minutes to obtain a homogeneous acetylation along the polymer chain. The medium is maintained and agitated for 4 hours.

The acetylation rate can be determined by elementary analysis. The non-acetylated monomer of the polymer (monomer B) presents a molar mass of 161.2 $gmol^{-1}$ ($C_6NO_4H_{11}$) while the acetylated monomer of the polymer (monomer A) presents a molar mass of 203.2 $gmol^{-1}$ (C_8NO5H_{13}). The elementary analysis of the polysaccharide obtained after step 2 is the following: C 39.22%; H 7.55%; and N 6.77%, which corresponds to an acetylation rate of 29% (x=0.29) confirmed by ^1H NMR.

In the third step, 2 L of the solution obtained after the second step, is placed under agitation. 120 g of DOTAGA anhydride is added and agitated for 16 hours. At the end of this reaction, the solution is diluted by 10 in ultra-pure water and purified by tangential purification using a membrane of 100 kDa. After a first step of dilution ending with a volume of 16 L, the solution is filtered with 480 L 0.1 M acetic acid maintaining a constant volume of 16 L, then with 320 L of ultra-pure water. This purification ends with a re-concentration to a volume of 8 L. HPLC-UV allows to verify that the DOTAGA unreacted has been successfully removed from the solution. The solution with a concentration of 10 g/L is then filtered using a nylon filter (0.4 um) before lyophilisation.

^1H NMR allows to determine the percentage of monomers functionalized with DOTAGA (y) on the polymer known to have an acetylation percentage, x.

The quantity of grafted DOTAGA is determined by spectrophotometric UV dosage using at 295 nm. MEX-CD1 contains 0.345 mmol of DOTAGA per gram of polymer.

The molar fraction of N-acetyl glucosamine repeat units x is x=0.29 using 1H-NMR, the molar fraction of the DOTAGA-grafted repeat units y is y=0.075 using 1H-NMR and dosage by copper using HLPC-SEC.

MEX-DTPA (Chitosan as polymer and DTPA as chelating moiety)

In the first step, 1 g of chitosan, 66 mL of ultra-pure water, and 0.84 mL of glacial acetic acid are introduced and agitated for 16 hours at a pH of 4.5 ± 0.5. A solution of pale yellow is observed.

In the second step, 20 mL of 1,2-propanediol is added to the pale yellow solution obtained from step 1 and agitated for 1 hour. A solution composed of 0.704 mL of acetic anhydride in 30 mL of 1,2-propanediol is slowly added over 10 minutes to obtain a homogeneous acetylation along the polymer chain. The medium is maintained and agitated for 4 hours.

In the third step, 0.8 g of DTPA bis-anhydride is added to the previously solution and agitated for 16 hours. At the end of this reaction, the solution is diluted by 10 in ultra-pure water and purified by tangential purification using a membrane of 100 kDa. After a first step of dilution ending with a volume of 1 L, the solution is filtered with 5 L 0.1 M acetic acid maintaining a constant volume of 1 L, then with 5 L of ultra-pure water. This purification ends with a re-concentration to a volume of ~100 mL. HPLC-UV allows to verify that the DTPA unreacted has been successfully removed from the solution.

The quantity of grafted DTPA is determined by spectrophotometric UV dosage using at 295 nm. MEX-DTPA contains 0.21 mmol of DTPA per gram of polymer.

The molar fraction of N-acetyl glucosamine repeats units x is x=0.58 using 1H-NMR, the molar fraction of the DTPA-grafted repeat units y is y=0.045 using 1H-NMR.

The MEX-DTPA as herein synthesized is useful for the treatment method as described in the present disclosure.

MEX-DOTAM (Chitosan as polymer and DOTAM as chelating moiety)

In the first step, 1 g of chitosan, 66 mL of ultra-pure water, and 0.84 mL of glacial acetic acid are introduced and agitated for 16 hours at a pH of 4.5 ± 0.5. A solution of pale yellow is observed.

In the second step, 20 mL of 1,2-propanediol is added to the pale yellow solution obtained from step 1 and agitated for 1 hour. A solution composed of 0.704 mL of acetic anhydride in 30 mL of 1,2-propanediol is slowly added over 10 minutes to obtain a homogeneous acetylation along the polymer chain. The medium is maintained and agitated for 4 hours. This solution is then purified by tangential purification using a membrane of 100 kDa, and 7 L ultra-pure water to remove the solvents and re-concentrated to ~100 mL. Using NaOH, the pH of this solution is raised to 7 ± 0.1. 8 mL of DMSO is added to the solution for an environment of 5% DMSO in water and agitated for 1 hour.

In the third step, 11 mL of a 100 g/L solution of DOTAM NHS-ester in DMSO is added over 10 minutes. Solution then diluted x2 with ultra-pure water and agitated for 16 hours. At the end of this reaction, the solution is diluted by 10 in ultra-pure water and purified by tangential purification using a membrane of 100 kDa. After a first step of dilution ending with a volume of 1 L, the solution is filtered with 5 L 0.1 M acetic acid maintaining a constant volume of 1 L, then with 5 L of ultra-pure water. This purification ends with a re-concentration to a volume of ~100 mL. HPLC-UV allows to verify that the DOTAM unreacted has been successfully removed from the solution.

The quantity of grafted DOTAM is determined by spectrophotometric UV dosage using at 295 nm. MEX-DOTAM contains 0.049 mmol of DOTAM per gram of polymer.

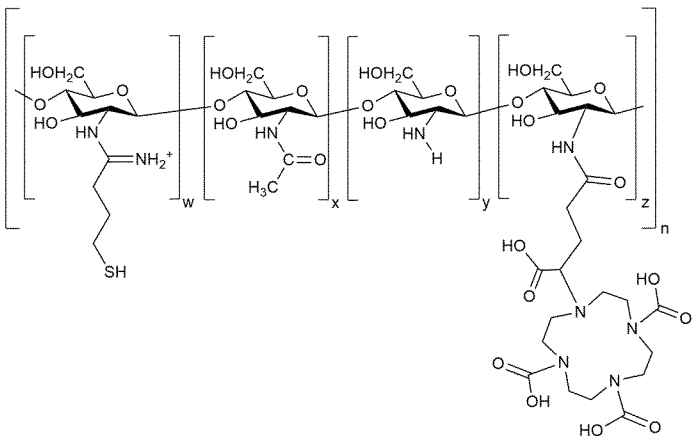
The molar fraction of N-acetyl glucosamine repeats units x is x=0.58 using 1H-NMR, the molar fraction of the DOTAGA-grafted repeat units y is y=0.0097 using 1H-NMR.

The MEX-DOTAM as herein synthesized is useful for the treatment method as described in the present disclosure.

MEX-CD-TBA (Chitosan as polymer, DOTAGA as chelating moiety and 4-thio-butyl-amidine as substituting moiety)

750 mg of MEX-CD1 (synthesis described above) are dissolved in 500 mL of ultrapure water in a 1 L round bottom flask and agitated for 2 hours. 50 mL of this solution are transferred in a 100 mL round bottom flask, then the pH is adjusted using a 10^{-1} M sodium hydroxide solution. 40 mg of 2-iminothiolane are added to the solution and left under stirring at room temperature for 48 hours. The mixture is then purified by tangential filtration using a 100 kDa cassette under a 150 mL/min flow: 50 mL of 5 mM hydrochloric acid, then the volume is lowered to 30 mL; the volume is then completed up to 100 mL and lowered to 30 mL twice. The same operation is performed one more time using a 5 mM and 1% NaCl solution. Finally, the same operation was repeated twice with MilliQ water. The polymer is lyophilized for further use.

The polymer obtained has the following structure:



EXAMPLE 1: Biodistribution of Orally Administered MEX-CD1 in Mice

MATERIALS AND METHODS:

Preparation of Solutions:

Solution 1: 10 g/L MEX-CD1

Lyophilized MEX-CD1 was dissolved in ultra-pure water at a concentration of 10 g/L.

Solution 2 : 5 g/L MEX-CD1 + 3.5 g/L NaCl

3.5 g/L NaCl was dissolved in ultra-pure water. Lyophilized MEX-CD1 was then dissolved in this solution with a final concentration of 5 g/L. This solution was sterilized at 121 ºC for 20 minutes.

Solution A: Concentrated Salt Solution for in vitro laboratory testing

263 g/L NaCl, 3.35 g/L KCl, 6.24 g/L CaCl2, 2.14 g/L MgCl2, 10.8 g/L Acetic Acid, 45 g/L Glucose in ultra-pure water

Solution B: 0.1 M Acetate Buffer, pH 4.6

The acetate buffer contains 11.4 mL of acetic acid (MS grade), 15.4 g of ammonium acetate, and 2 L of ultra-pure water.

Experimental Protocols:

Protocol 1: High Pressure Liquid Chromatography – Mass Spectroscopy (HPLC-MS)

The eluent used for this method was acetate buffer (solution B, prepared with MS-Grade certified products), le injection volume was 10 uL, and the flow rate was fixed at 0.4 mL/min. The column used was a SEC Polysep GFC-P 4000 series. The HPLC is coupled to a Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for the detection of chosen isotopes.

Protocol 2: Vivaspin Ultrafiltration Experimentation

20 mL Vivaspin Centrifugation tubes with an internal membrane of 30 kDa were used. The centrifugation was performed at 4000 rpm for 10-30 minute cycles at a time. After each centrifugation, the solution that had passed through the membrane was collected for each sample, henceforth referred to as the undernatant.This was performed in one passage with ideally ~0.5 mL of supernatant remaining and collected for ICP-MS analysis.

The samples, including the original solution, the supernatant, and the undernatant, were analyzed by ICP-MS to determine the concentration of metals of interest within each solution. The samples were diluted by a factor of (at least) 10 using 1% HNO3. An internal standard (indium) was added to each sample with a final concentration of 2 ppb In. The analyses were performed in either KED (Kinetic Energy Discrimination) mode, which introduced a flux of helium within the chamber, or standard mode. KED was employed with

the analysis was coupled with other analyses of more sensitive elements, but the majority of the samples were done in standard mode for: 208Pb, 206Pb, 111Cd, 112Cd, 114Cd.

A calibration curve ranging from 0.01 to 10 ppb for lead and cadmium was created to allow the system to convert the counts per second recorded for each sample into a concentration in ppb.

0.2 mL of solution 4 was orally administered for 8 mice. 2 mice were sacrificed at each time point: 1, 2, 4, and 24 hours. The fluorescence study was performed using a CCD camera and the following parameters: 2 sets of spotlights for excitation at 633 and 470 nm and 2 sets of filters at 680 ± 20 and 520 ± 20 nm, then the organs were digested in nitric acid for gadolinium quantification using ICPMS.

Results

Qualitative biodistribution with fluorescence

No fluorescence is observed at any time point within Kidneys, Liver, Brain, Spleen, Heart, Urine, Lungs, Bone, Skin, Blood, & Muscle after 1, 2, 4, and 24 h. Fluorescence is only observed within the Stomach, Intestines, and Colon after 1, 2, and 4 hours. A small level of autofluorescence is seen within the stomach of the control mouse.

The fluorescence results show the presence of MEX-CD1 within only the stomach, intestines, and colon (digestive tract) (table 1).

Organ	Sacrifice Time	Range of Fluorescence Intensity of Cyanine5.5 (x1000)
Stomach	1 hour	817.8 – 1210.7
	2 hours	350.6 – 1301.3
	4 hours	575.3 – 652.8
	24 hours	66.0 – 990.4
	Control	278,3
Intestine	1 hour	311.2 – 314.0
	2 hours	485.4 – 584.4
	4 hours	66.9 – 516.3
	24 hours	0.3 – 178.4
	Control	6,4
Colon	1 hour	3.5 - 76.4
	2 hours	154.0 – 203.0
	4 hours	167.2 – 615.8
	24 hours	96.6 – 126.0
	Control	184,9

Some auto-fluorescence is observed within the stomach of the control mouse which explains the remaining fluorescence at 24 hours within the two stomachs.

Quantitative Biodistribution with ICP-MS

As shown in figure 1, each time point had two experimental mice, therefore the average percent gadolinium was taken. This was then plotted with the standard deviation in Figure 1. The trend shows that within the first hour, the majority of MEX-CD1 is already found within the intestine and at 24 hours, all of the MEX-CD1 is eliminated from the digestive system of the mice.

Other organs were also assessed, in which all presented less than 1% of the administered gadolinium in concentration and was therefore considered as zero.

Therefore, orally administered MEX-CD1 remains within the digestive tract, does not pass the intestinal membrane into the blood, and is completely eliminated after one day.

EXAMPLE 2:

Materials and methods:

Preparation of Solutions:

Solution 1: 10 g/L MEX-CD1

Lyophilized MEX-CD1 was dissolved in ultra-pure water at a concentration of 10 g/L.

Solution 2: 10 g/L chitosan

Chitosan (of medical grade and animal origin; weight and number average molar masses w = 2.583*105 g/mol, Mn = 1.323*105 g/mol respectively; acetylation degree = 6 ± 0.5%) was dissolved in ultra-pure water and acetic acid (pH = 4.6) at a concentration of 10 g/L.

Solution 3 : 10 g/L MEX-CD1 sterilized

Lyophilized MEX-CD1 was dissolved in in ultra-pure water at a concentration of 10 g/L. This solution was sterilized at 121 ºC for 20 minutes.

Solution 4 : 3.5 g/L NaCl sterilized

3.5 g/L NaCl was dissolved in ultra-pure water. Lyophilized MEX-CD1 was then dissolved in this solution with a final concentration of 5 g/L. This solution was sterilized at 121 ºC for 20 minutes.

***Solution A**: 0.1 M Acetate Buffer, pH 4.6*

The acetate buffer contains 11.4 mL of acetic acid (MS grade), 15.4 g of ammonium acetate(MS grade), and 2 L of ultra-pure water.

Experimental protocols:

Protocol 1: High Pressure Liquid Chromatography – Mass Spectroscopy (HPLC-ICP-MS)

The eluent used for this method was acetate buffer (solution A, prepared with MS-Grade certified products), the injection volume was 10 µL, and the flow rate was fixed at 0.4 mL/min. The column used was a SEC Polysep GFC-P 4000 series. The HPLC is coupled to a Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for the detection of chosen isotopes.

Protocol 2: Vivaspin Ultrafiltration Experimentation

20 mL Vivaspin Centrifugation tubes with an internal membrane of 50 kDa were used. The centrifugation was performed at 4000 rpm for 10-30 minute cycles at a time. After each centrifugation, the solution that had passed through the membrane was collected for each sample, henceforth referred to as the undernatant, and the tubes were refilled with the original solution. This was performed in cycles until the original solution was passed through the membrane in totality. Ideally, ~1 mL of supernatant remained and was collected for ICP-MS analysis.

The samples, including the original solution, the supernatant, and the undernatant, were analyzed by ICP-MS to determine the concentration of metals of interest within each solution. The samples were diluted by a factor of 10^4 for the undernatant and the original solutions and of 10^5 for the supernatants using 1% HNO_3. An internal standard (indium) was added to each sample with a final concentration of 2 ppb In. The analyses were performed in standard mode.

A calibration curve ranging from 0.001 to 0.2 ppb for Cs, Ir, Th, Tl, Sr and U was created to allow the system to convert the counts per second recorded for each sample into a concentration in ppb.

1.Orally Administered MEX-CD1 in Mice Exposed to uranium

The study was performed on C57Bl/6 young male mice. Mice were gavaged daily with solution 3 in the volume to achieve 50 mg/kg dose of MEX-CD1 for 7 days. On the 4th day they were given orally with the saline solution of uranium salt $UO_2(NO_3)_2$*$6H_2O$ in the volume to achieve the dose of 50 mg/kg of pure uranium. The overall dose of uranium was split on 2 administrations 4 h in between. There were also three control groups:

1) Mice given with solution 4 instead of all tested substances (**healthy control**);
2) Mice given with MEX-CD1 and with solution 4 instead of uranium salt (**MEX-CD1 contro**l - to exclude any MEX-CD1 toxicity);

3) Mice given with uranium salt and solution 4 instead of MEX-CD1 (**positive control** – mice poisoned with U and obtained no treatment).

At the 8th day all the animals were sacrificed, blood, kidneys and spleen were collected for uranium detection, biochemical and hematological studies. During the study mice were daily observed and weighed as well.

Results

Body Weight Changes *over 8-day, daily administration of MEX-CD1 and/or uranium in mice*

There was no significant bodyweight change for the MEX-CD1 and healthy control groups (figure 2), which supports the harmless nature of MEX-CD1. For the 2 groups where uranium was administered, a bodyweight loss caused by uranium toxicity was evidenced; however, MEX-CD1 seems to speed up the bodyweight recovery of the mice, as can be observed within the last three days of the study (figure 3).

Uranium blood and tissue content after oral administration of MEX-CD1 and/or uranium in mice

Uranium was not detected in blood at the 4th day after administration (8th day of the study), as we suggested according to the literature Bing, D.; Jing-Wei, H.; Lian, D.; Liang, D. Biodistribution of Uranium in Mice and Influencing Factor Preliminary Discussion. *Asian J. Chem.* **2014**, *26* (13), 3932–3936. https://doi.org/10.14233/ajchem.2014.16043. However, Uranium was accumulated in kidney and spleen, and MEX-CD1 partially (in kidneys) or completely (in spleen) prevented its accumulation in these organs (figure 4). These results show a reduced amount of uranium content in both kidneys and spleen (significant for spleen) after administration of MEX-CD1, which is a first sign that it can mitigate uranium toxicity in vivo. Partial preservation of uranium in kidney could be explained by its predominant and substantial accumulation in this organ.

Hematology after 8-day, daily administration of MEX-CD1 and/or uranium in mice

Acute uranium poisoning is evidenced in some hematological parameters among the saline-treated mice (see figure 5); these changes are indicators of inflammatory processes. For all of these hematological parameters, there was no change after administration of MEX-CD1

alone (MEX-CD1 control) relatively to healthy control, so this provides evidence that there is no negative impact of this treatment on hematological parameters. Besides, the figure above shows in particular that the white blood cell (WBC) and platelet (PLT) counts are everted to normal levels with the oral administration of MEX-CD1.

Creatinine levels after 8-day, daily administration of MEX-CD1 and/or uranium in mice.

Serum creatinine level is an indicator of kidney function; uranium acute poisoning has been linked with an increase of creatinine level in plasma (Vicente-Vicente, L.; Quiros, Y.; Pérez-Barriocanal, F.; López-Novoa, J. M.; López-Hernández, F. J.; Morales, A. I. Nephrotoxicity of Uranium: Pathophysiological, Diagnostic and Therapeutic Perspectives. *Toxicological Sciences* **2010**, *118* (2), 324–347. https://doi.org/10.1093/toxsci/kfq178). The results displayed on figure 6 show that creatinine is significantly reverted to its normal levels after administration of MEX-CD1.

2. In Vitro Chelation of cobalt and thorium chelation by MEX-CD1 using HPLC-SEC-ICP-MS

Results

Cobalt chelation by MEX-CD1 in solution A

Using a metal mixture of Co, Th, Tl, Cs, Sr, Ir, U with a concentration of either 140 ppb or 2800 ppb in 1% HNO3 prepared using the metal standards purchased from SCP Science, either solution 1 or solution 2 and solution A the following 13 samples were created and analyzed by HPLC-MS following Protocol 1.

Sample	Polymer	Metal concentration (ppb)
0	1 g/L MEX-CD1	0
1		0.25
2		0.5
3		2.5
4		5
5		12.5
6		25
7		37.5
8		50
9		62.5
10		75
11		87.5
12		100

19	1 g/L chitosan	25
20		37.5
21		50
22		75
23		87.5
24		100

The chromatograms of MEX-CD1, show a linear increase of the peak area at 15 minutes (specifically corresponding to the polymer) with cobalt concentration (data not shown). Conversely, the chromatograms of chitosan, and metal solutions do not show any peak at this retention time, data not shown).

This demonstrates an ability for MEX-CD1 to chelate cobalt from a concentration of at least 25 ppb at pH = 4.5.

Thorium chelation by MEX-CD1 in solution A

Using a solution of 5 ppm Th in 1% HNO_3 prepared using the 1000 ppm metal standard (SCP Science), solution 1 and solution A the following 9 samples were created and analyzed by HPLC-MS following Protocol 1.

Sample	**Polymer**	**Thorium concentration (ppb)**
55	1 g/L MEX-CD1	50
56		75
57		100
58		125
59		150
60		175
61		200
62		250
63		500

The results show a linear increase of the peak area at 15 minutes (specifically corresponding to the polymer) with thorium concentration, with a slope break after 200 ppb. This proves that MEX-CD1 (1 g/L) is able to chelate thorium from a concentration of at least 50 ppb at pH = 4.6 (data not shown).

3. *In Vitro* capture of strontium, cesium, iridium, thallium, thorium and uranium by MEX-CD1 at different pH using Vivaspin and ICP-MS

Using the metal standards purchased from SCP Science with concentrations of 1000 ppm of Th, Tl, Cs, Sr, or U in 5% HNO3 or 1000 ppm of Ir in 10% HCl as well as solution 1, 9 samples were created as follows in table 2:

Table 2:

Sample	Metal concentration (ppm)	MEX-CD1 concentration (g/L)	pH
1	2	0	1
2	2	0	5
3	2	1	1
4	2	1	2
5	2	1	3
6	2	1	4
7	2	1	5
8	2	1	6
9	2	1	7

The pH was adjusted using 1 M NaOH and 1 M HCl. Following protocol 2, the samples were experimentally analyzed. The following table 3 summarizes the percentage in mass of each metal captured by the polymer.

Table 3:

		Percentage of metal captured					
Sample	**pH**	**Cs (I)**	**Ir (III)**	**Th (IV)**	**Tl (I)**	**Sr (II)**	**U (VI)**
Metal only	1	2%	5%	6%	2%	2%	3%
Metal only	5	4%	7%	100%	2%	0%	4%
1 g/L MEX-CD1 + 2 ppm metal	1	9%	22%	**57%**	11%	0%	16%
1 g/L MEX-CD1 + 2 ppm metal	2	5%	20%	**93%**	5%	-4%	11%
1 g/L MEX-CD1 + 2 ppm metal	3	2%	14%	**96%**	2%	12%	19%
1 g/L MEX-CD1 + 2 ppm metal	4	19%	30%	**97%**	19%	29%	**59%**
1 g/L MEX-CD1 + 2 ppm metal	5	14%	28%	**94%**	16%	**69%**	**70%**
1 g/L MEX-CD1 + 2 ppm metal	6	22%	28%	**95%**	29%	**93%**	**83%**
1 g/L MEX-CD1 + 2 ppm metal	7	**51%**	**54%**	**97%**	**56%**	**95%**	**93%**

This percentage $\%_{captured}$ has been calculated using the metal mass in the undernatants m_{UN} and the initial metal mass in the solutions m_i as described by the following formula.

$$\%_{captured} = 1 - \frac{m_{UN}}{m_i}$$

As the volume of the supernatants considerably decreased, their measurement was less precise than that of the undernatants. Besides, the amount of metal in the supernatants can be biased by the adhesion of the polymer onto the membrane; consequently, the most reliable results are the analysis of the undernatants (even though the supernatants generally follow the same trend). For all of the metals analyzed, there is a significant decrease in metal amount in the undernatant at pH = 7 and even at lower pHs for Sr, U and Th.

The figure 7 displays the percentage of metal amount captured by MEX-CD1 using the analysis of the undernatants (see previous table 3). It shows an increase of this percentage with pH for all metals (Cs, Th, Tl, Ir, Sr, U), proving the efficacy of MEX-CD1 to capture significantly these metals and prevent them from crossing the membrane.

EXAMPLE 3: protection of intestinal cells from reactive oxygen species by MEX-CD1

For this study, the caco2 cell line is used as a model of the intestinal epithelial barrier. The cells are grown in MEM medium with 20% fetal bovine serum. The cells are incubated at 37°C and passed every 5 days. Confluency is measured every 6 hours for 3 days using a CellCyte X™ live cell imaging device from CYTENA. Cell death is evaluated using the same device and the propidium iodide (PI) fluorescent probe (Exc=580-598 nm; Em=612-680 nm).

1. Study of MEX-CD1 toxicity in caco2 cells

The cells are treated with increasing doses of MEX-CD1 (0.5 g/L, 1 g/L, 2 g/L, 5 g/L in culture medium) in 96-wells plates. Negative (non-treated) and positive (using oxaliplatin) controls are also performed(data not shown for the positive control). For each condition, at least 6 replicates were performed. The death index (indicator of cellular death; death index = PI/confluency) was calculated.

Figure 8 displays the evolution of the death index within 78 hours after treatment.

There is no toxicity of MEX-CD1 for concentrations of 2 g/L or below; significant death appears at 5 g/L. Following these results, the concentration of 2 g/L has been identified as the limit dose of toxicity for further studies.

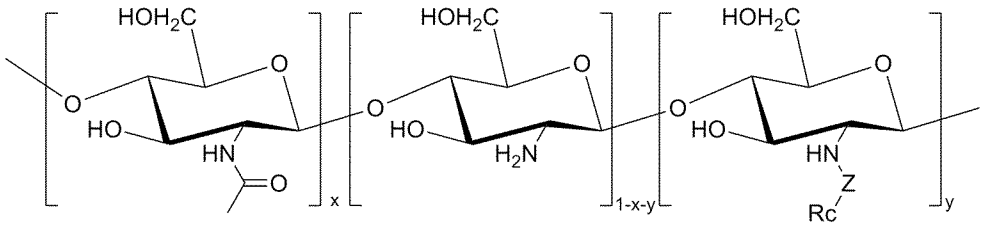
2. *Study of caco2 cells protection by MEX-CD1 against H_2O_2-induced oxidative stress*

Caco2 cells were incubated using 4 different conditions: non-treated, treated with 2 g/L MEX-CD1, treated with 1 mM H_2O_2, and treated with 2 g/L MEX-CD1 and 1 mM H_2O_2. Figure 9 displays the death index for all of this conditions.

It shows that there is a significant decrease of death index in the H_2O_2-treated cells after administration of 2 g/L MEX-CD1, which indicates that MEX-CD1 has a high potential in protecting the cells from oxidative stress.

CLAIMS

1. A functionalized polymer, for use in a method for treating radioactive contamination, said method comprising orally administering, in a subject at risk or suspected of radioactive contamination, an efficient amount of a functionalized polymer, wherein said functionalized polymer is a functionalized statistic chitosan of weight average molecular mass between 100kDa and 1000kDa and of formula (I):



(I)

wherein

each Rc is a chelating moiety,

each Z is independently a linker which is a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

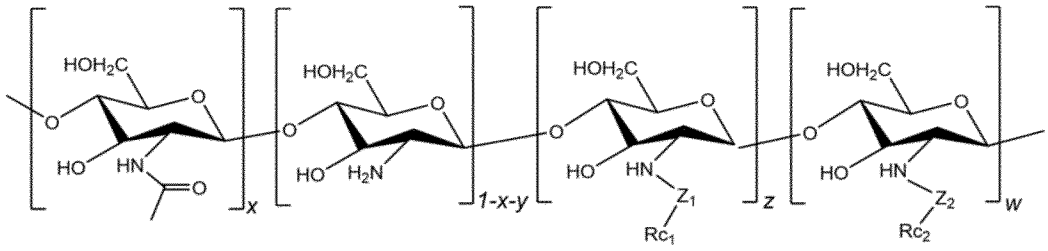
x is between 0.005 and 0.7, preferably between 0.05 and 0.7,

y is between 0.005 and 0.5, preferably between 0.01 and 0.2,

the ratio y/x being greater than or equal to 0.01, preferably greater than or equal to 0.02, and

the sum x + y being greater than or equal to 0.15, preferably greater than or equal to 0.30.

2. A functionalized polymer, for use according to Claim 1, wherein said functionalized polymer is a functionalized statistic chitosan of formula (II):



(II)

wherein

Rc_1 is a chelating moiety and Rc_2 is a different chelating moiety or ROS scavenger moiety ,

Z_1 and Z_2, identical or different, are linkers which are a single bond or a hydrocarbon chain containing between 1 and 12 carbon atoms, said hydrocarbon chain is linear or branched and optionally contains one or more unsaturations and one or more heteroatoms, preferably chosen from nitrogen, oxygen, sulfur and halogens,

x is between 0.005 and 0.7, preferably between 0.05 and 0.7, and more preferably between 0.2 and 0.6,

y=z+w is between 0.01 and 0.7, preferably between 0.05 and 0.3,

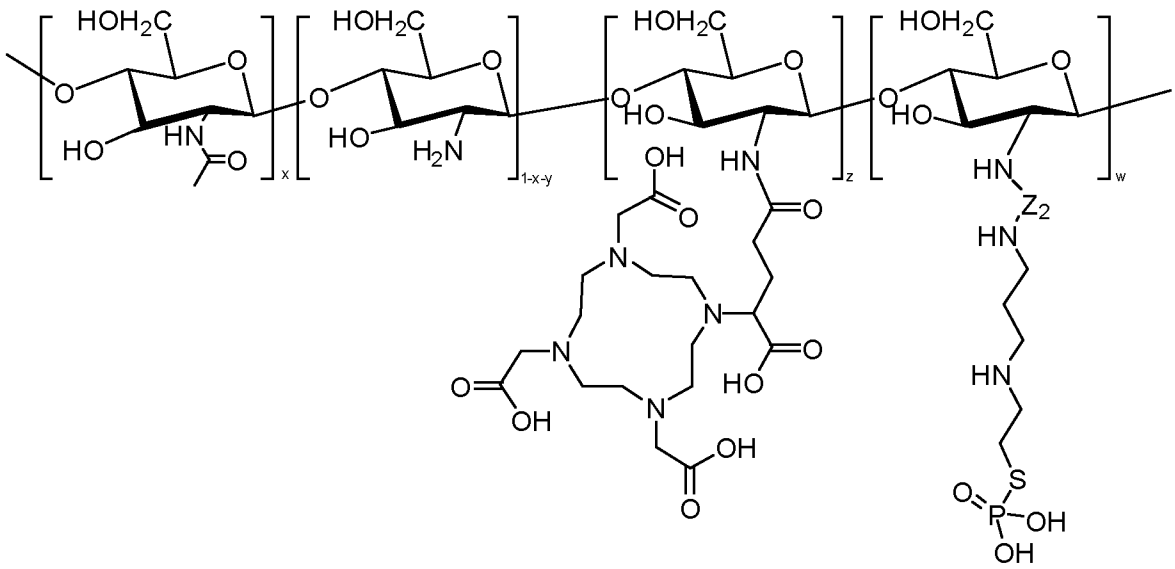
the ratio y/x being greater than or equal to 0.05, preferably greater than or equal to 0.15,

the sum x + y being greater than or equal to 0.15, preferably greater than or equal 0.30, more preferably greater than or equal to 0.35.

3. The functionalized polymer of formula II, for use according to Claim 2, wherein x is between 0.005 and 0.6; y is between 0.1 and 0.9; the ratio y/x being greater than 0.3; and z is between 0.5 and 1.

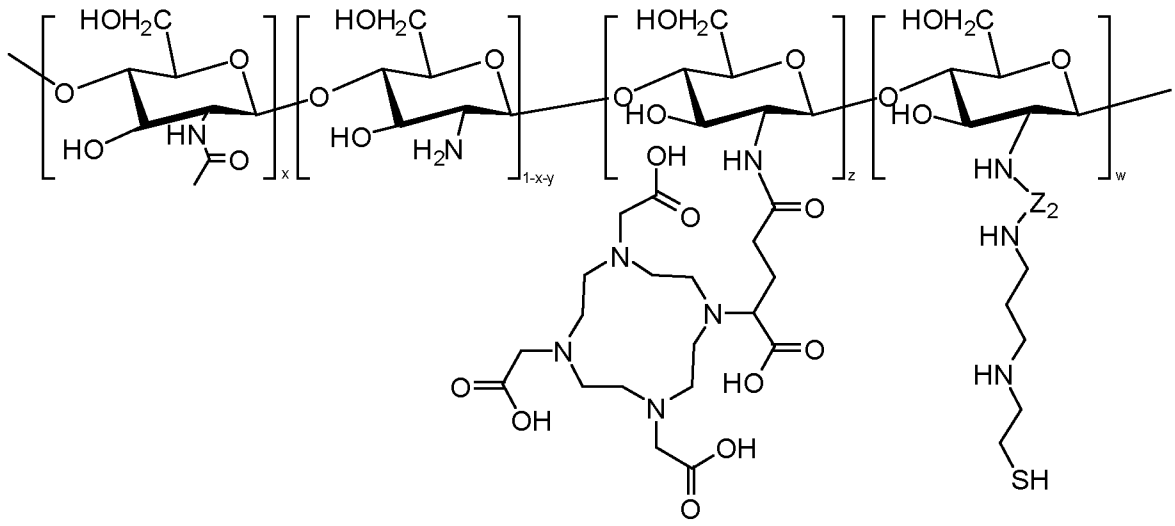
4. The functionalized polymer of formula II, for use according to Claim 2 or 3, wherein Rc2 is a ROS scavenger moiety independently selected from the group consisting of 4-thio-butyl-amidine, amifostine, WR-1065, lipoic acid, genistein, apigenin, N-acetylcysteine, cysteine, cysteamine, vitamin A, beta-carotene, vitamin C, vitamin E, resilisib.

5. The functionalized polymer of formula II, for use according to any one of Claim 2 to 4, wherein said functionalized polymer is a functionalized statistic chitosan of formula (V) or (VI):



(V)

wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12,



(VI)

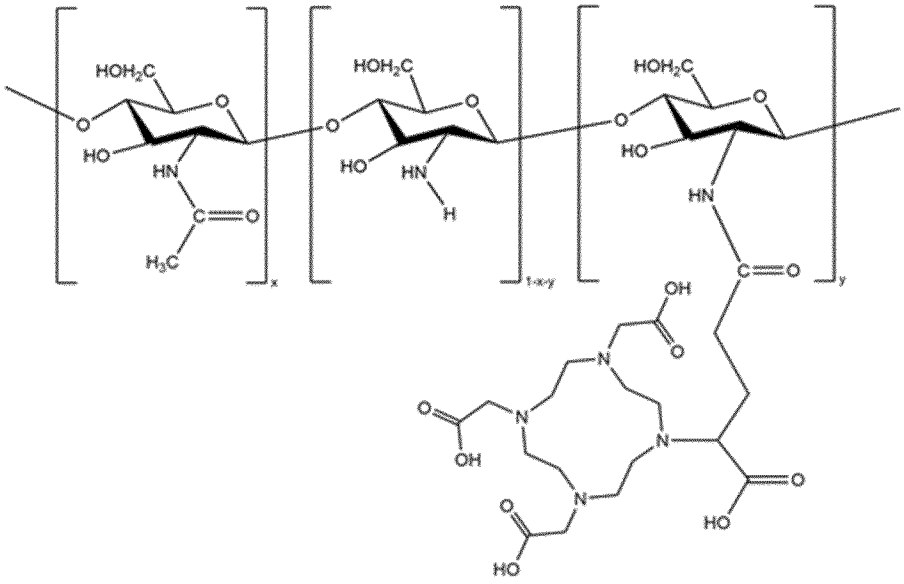
wherein x is between 0.2 and 0.6, more preferably x is between 0.25 and 0.4, typically about 0.3, and y=z+w is between 0.05 and 0.3, more preferably between 0.1 and 0.2, typically about 0.12.

6. The functionalized polymer for use according to any one of claims 1-5, for preventing from internal contamination by a radiocontaminant.

7. The functionalized polymer for use according to any one of claims 1-6,, which further acts as a radical oxygen species (ROS) scavenger for reducing toxicity associated to external radioactive contamination.

8. The functionalized polymer for use according to any of claims 1-7, wherein said radiocontaminant is a radionuclide selected from a radioactive isotope of Uranium, Thorium, Thalium, Cesium, Strontium, Iridium and Cobalt, or mixtures thereof.

9. The functionalized polymer for use according to any one of claims 1-8, having the following formula (III):



(III)

wherein x is between 0.25 and 0.4, typically about 0.3, and y is between 0.05 and 0.2, typically about 0.07.

10. The functionalized polymer for use according any to one of claims 1 to 9, wherein the functionalized polymer is first administered at least 6 hours, preferably at least 12 hours prior to first exposure to said radiocontaminant.

11. The functionalized polymer for use according to any one of Claims 1 to 10, wherein said subject will be, is, or has been exposed to radioactive contamination due to accidents involving nuclear reactors.

12. The functionalized polymer for use according to any one of Claims 1 to 11, wherein said subject will be, is, or has been exposed to radioactive contamination due to presence of said subject in a zone during or after an accident involving a nuclear reactor.

13. The functionalized polymer for use according to any one of Claims 1 to 12, wherein said subject is at risk of exposure to radioactive contamination due to presence in a

combat zone during or after the use of a nuclear explosive or a radiological dispersal device.

Fig 1

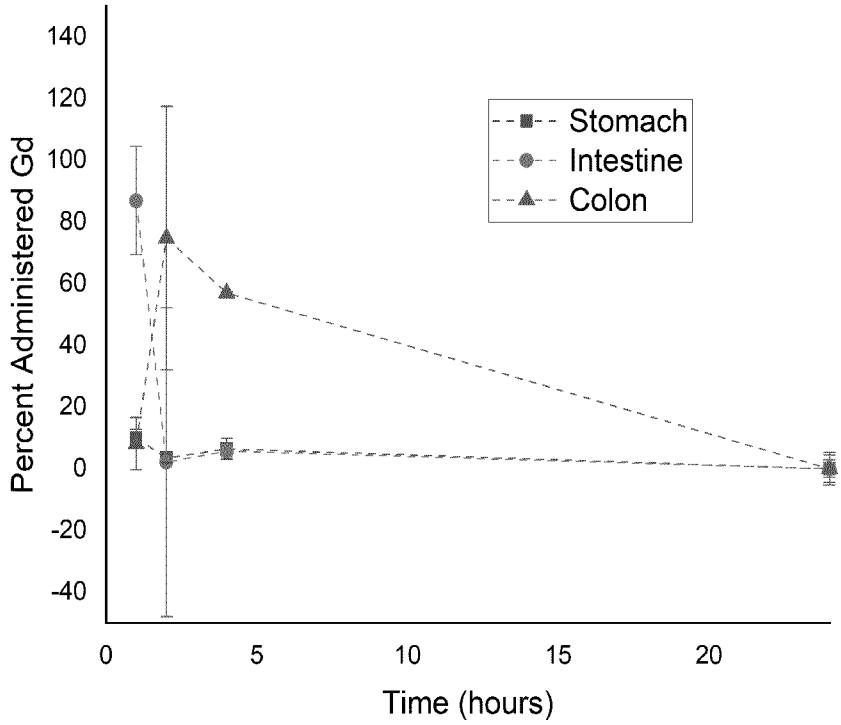

Percent Administered Gd
140
120
100
80
60
40
20
0
-20
-40
0
5
10
15
20
Time (hours)
Stomach
Intestine
Colon

Fig 2

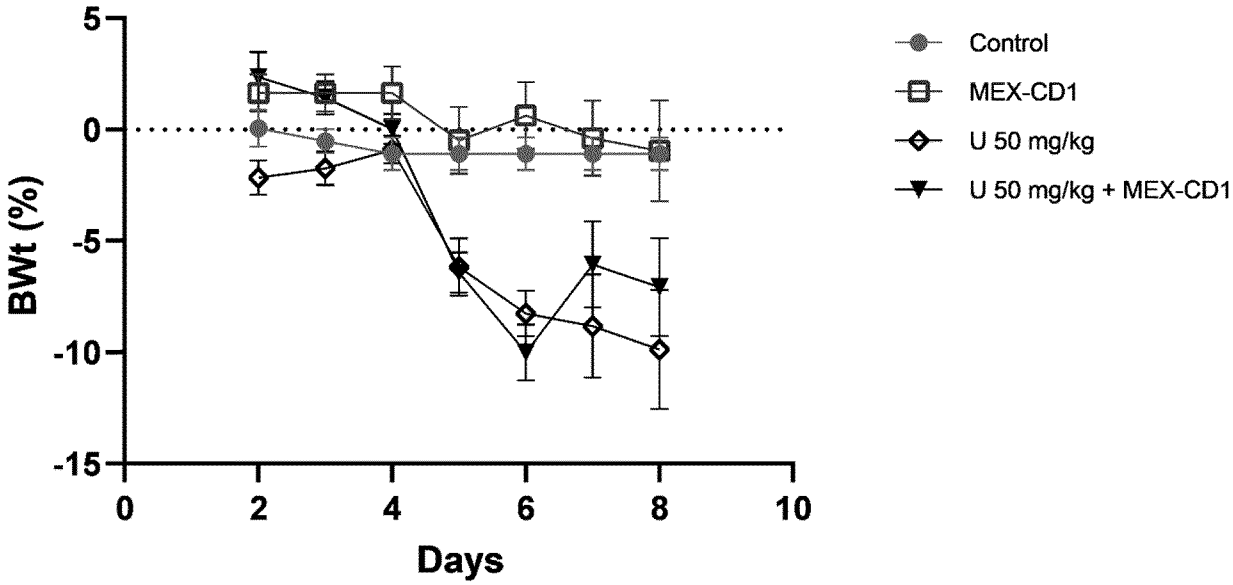

BWt (%)
5
0
-5
-10
-15
0
2
4
6
8
10
Days
Control
MEX-CD1
U 50 mg/kg
U 50 mg/kg + MEX-CD1

Fig. 3

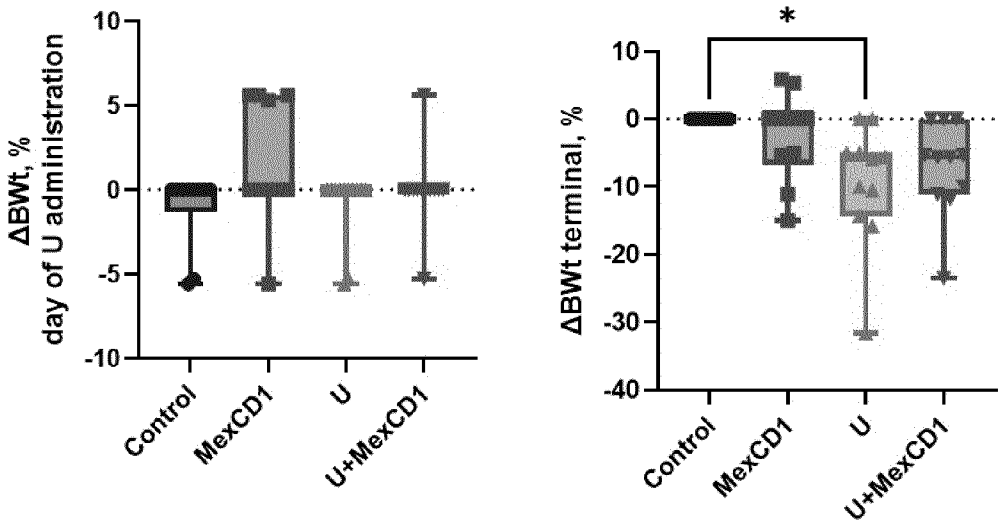

ΔBWt, %
day of U administration
10
5
0
-5
-10
Control
MexCD1
U
U+MexCD1
*
ΔBWt terminal, %
10
0
-10
-20
-30
-40
Control
MexCD1
U
U+MexCD1

Fig 4

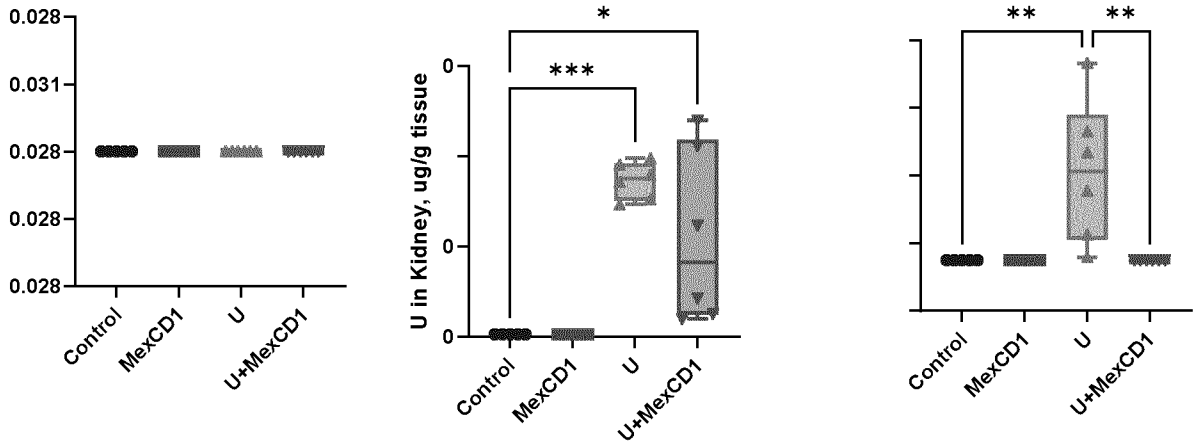

0.028
0.031
0.028
0.028
0.028
Control
MexCD1
U
U+MexCD1
*
***
U in Kidney, ug/g tissue
0
0
0
Control
MexCD1
U
U+MexCD1
**
**
Control
MexCD1
U
U+MexCD1

Fig 5

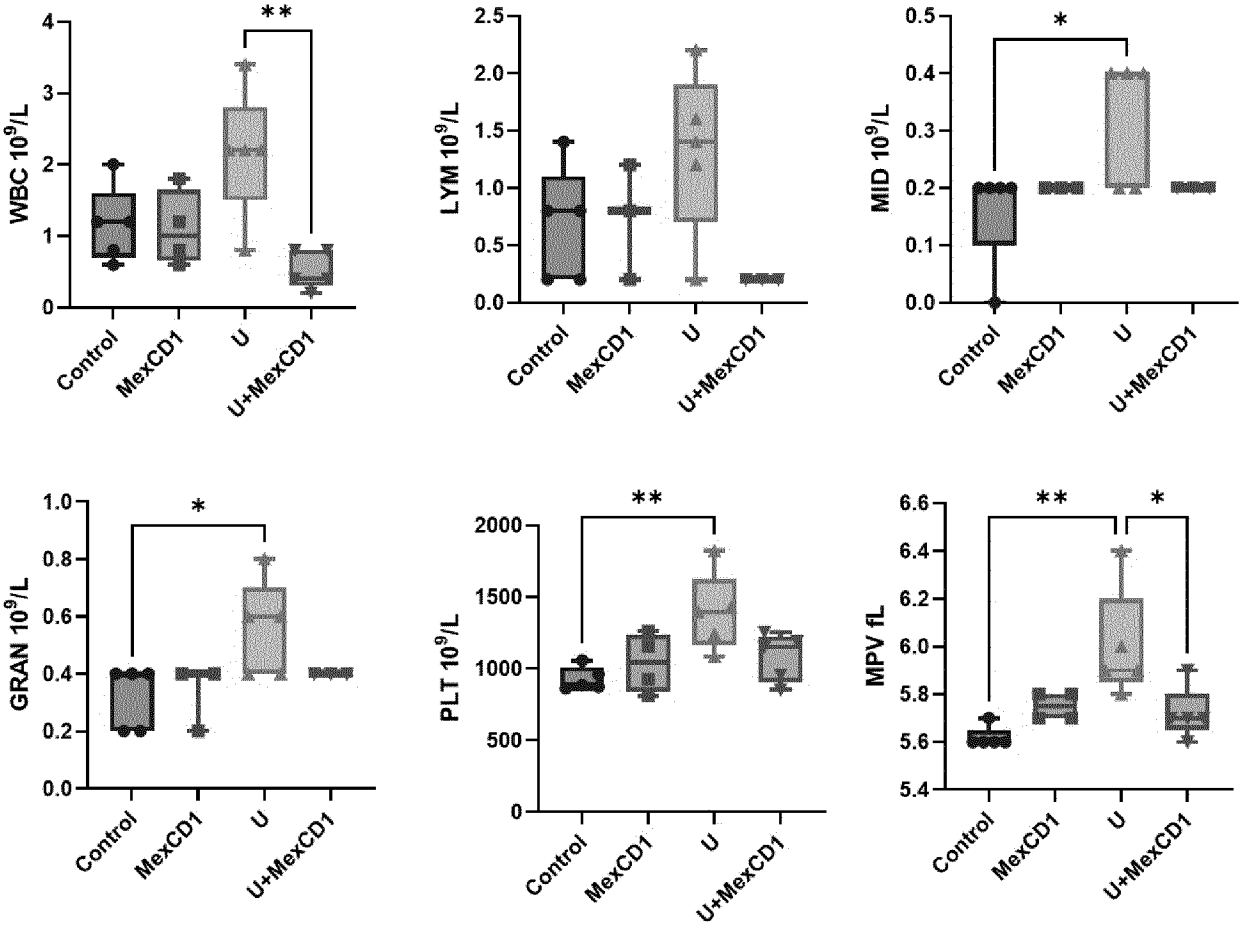

WBC 10^9/L
LYM 10^9/L
MID 10^9/L
GRAN 10^9/L
PLT 10^9/L
MPV fL
Control
MexCD1
U
U+MexCD1

Fig 6

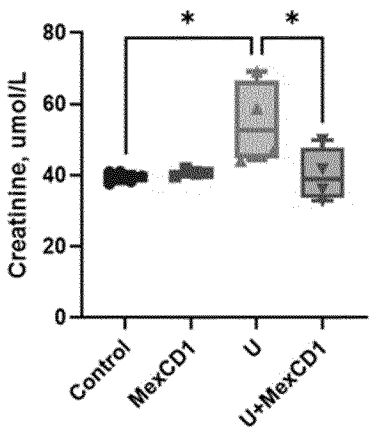

Creatinine, umol/L
Control
MexCD1
U
U+MexCD1

Fig 7

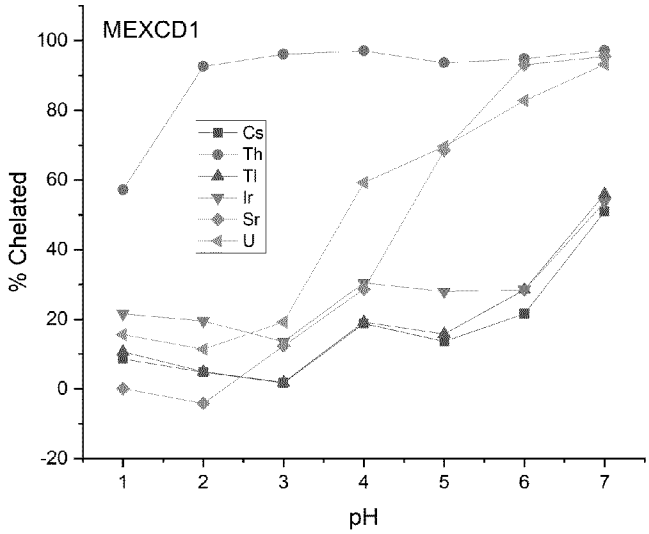


Fig 8

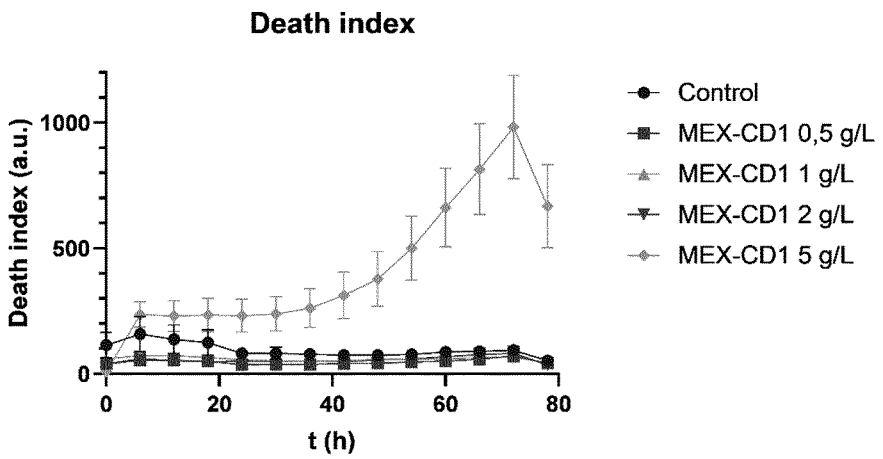
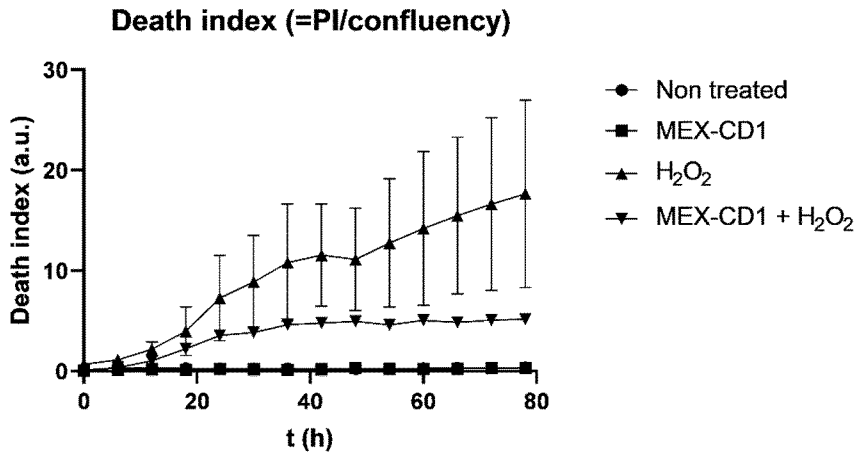


Fig 9



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/068229

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/61 A61P39/04 A61P39/06
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 115 177 738 A (FIRST AFFILIATED HOSPITAL ARMY MEDICAL UNIV OF PLA) 14 October 2022 (2022-10-14)	1-3,6,7, 9,10
A	paragraph [0003] - paragraph [0014] examples 1-3	4,5,8, 11-13

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2024	30/09/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Monami, Amélie

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/068229

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Howard Jordyn Ann ET AL: "Combating lead and cadmium exposure with an orally administered chitosan-based chelating polymer", Scientific reports, 7 February 2023 (2023-02-07), XP093106126, DOI: 10.1038/s41598-023-28968-4 Retrieved from the Internet: URL:https://www.nature.com/articles/s41598-023-28968-4.pdf [retrieved on 2023-11-27] cited in the application	1-3,6,7, 9,10
A	abstract figure 1a page 2, paragraph 8 - page 3, paragraph 1 page 10, paragraph 8 - paragraph 12	4,5,8, 11-13
A	WO 2022/023677 A1 (MEXBRAIN [FR]; CENTRE NAT RECH SCIENT [FR] ET AL.) 3 February 2022 (2022-02-03) cited in the application paragraph [0001] examples 1-8 paragraph [0030] - paragraph [0037]	1-13
A	KR 101 690 179 B1 (INDUSTRY-ACADEMIC COOP FOUND OF SUNCHON NAT UNIV [KR]) 28 December 2016 (2016-12-28) paragraph [0001] - paragraph [0005] paragraph [0059] paragraph [0064] - paragraph [0090] figures 1-4	1-13
A	LEVITSKAIA TATIANA G ET AL: "Investigation of chitosan for decorporation of 60Co in the rat", HEALTH PHYSICS, LIPPINCOTT, WILLIAMS & WILKINS, HAGERSTON, MD, USA, vol. 97, no. 2, 1 August 2009 (2009-08-01), pages 115-124, XP008125268, ISSN: 0017-9078 abstract page 116, column 2, paragraph 1 page 123, column 2, paragraph 1 - paragraph 3	1-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/068229

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 4188966 A1	07-06-2023
		FR 3112943 A1	04-02-2022
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KR 101690179 B1	28-12-2016	NONE	