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(54) Title: BISMUTH METALLIC (0) NANOPARTICLES, PROCESS OF MANUFACTURING AND USES THEREOF

(57) Abstract: The present invention relates to a process for manufacturing stable and monodisperse biocompatible bismuth metallic (0) nanoparticles, in aqueous solution, using a water-soluble bismuth precursor, a water-soluble reducing agent and a coating agent. The process can be carried out in continuous flow. The invention also relates to corresponding stable biocompatible bismuth metallic (0) nanoparticles having hydrophilic, hydrophobic or fluorophilic properties and uses thereof, especially medical applications thereof such as theranostic applications.

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BISMUTH METALLIC (0) NANOPARTICLES, PROCESS OF MANUFACTURING AND USES THEREOF

FIELD OF INVENTION

5 The present invention pertains to the field of nanoparticles, especially bismuth metallic (0) nanoparticles. In particular, the invention relates to an efficient process for manufacturing, in aqueous solution, stable and monodisperse biocompatible bismuth (0) nanoparticles. The process of the invention can be carried out in continuous flow. The invention also relates to corresponding bismuth (0) nanoparticles having hydrophilic,
10 hydrophobic or fluorophilic properties and uses thereof, especially medical applications thereof.

Hereafter, bismuth metallic (0) nanoparticles are also referred to as bismuth (0) nanoparticles or more simply as bismuth nanoparticles (Bi NPs).

15 BACKGROUND OF INVENTION

Bismuth is a diamagnetic semimetal showing several properties such as high magnetoresistance, thermal conductivity and high anisotropic electronic behavior. Bi NPs may thus have electronic applications and are also studied as chemical catalysts.

20 Bismuth element is further characterized by the highest X-ray absorption compared to other heavy metals. As bismuth is biocompatible and may be administrated at high dose, Bi NPs have very interesting medical applications, for example as radiosensitizers in radiotherapy, for photothermal therapy or as contrast agents for X-ray, photoacoustic, photothermal or fluorescence medical imaging.

25 The aim of radiotherapy is to deliver a lethal dose of radiations to tumor cells while avoiding healthy tissues. One approach to increase the sensitivity of cancerous cells to radiation is to use heavy-element (high Z) nanoparticles targeting cancerous cells as radiosensitizers. Indeed, elements such as gold, silver, platinum or bismuth, incorporated

to nanoparticles have a large cross-section for X-ray absorption and photoelectron generation. Such nanoparticles thus significantly increase deposited dose in their vicinity because of their high-energy absorption coefficients.

In a pioneering work, Hossain described a technique based on Bi NPs enabling an early
5 detection and eradication of circulating tumor cells (Hossain et al., *Biosensors and Bioelectronics*, Vol. 38, 2012, pp. 348-354). In this work, Bi NPs were conjugated with folic acid to target folate receptors overexpressed on cancer cells surface. These Bi NPs enabled detecting circulating tumor cells by X-ray fluorescence and selectively killing these cells by X-ray radiations. Hossain also evaluated performance of nanoparticles
10 series (bismuth, gold and platinum) as radiosensitizers by mathematical modelization (Hossain et al., *The Journal of Physical Chemistry*, Vol. 116, 2012, pp. 23047-23052). According to this modelization, Bi NPs should provide higher dose enhancements than Au and Pt NPs for a given nanoparticle size, concentration and location.

Following the same physical principle, nanoparticles of heavy elements may be used as
15 radiosensitizers to eliminate bacteria. Free radicals and photoelectrons, generated by efficient X-ray irradiation of nanoparticles, contribute to significant DNA damage in bacteria.

Besides, the potential of radiopaque nanoparticles was recently explored as high contrast, long circulating X-ray contrast agents (XCA). Especially, nanosized contrast agents are
20 proposed for vessel imaging since nanoparticles increase blood residence time by limiting the “leakage” across the capillary vessels. Bismuth compounds are attractive to design such XCA since Bi has a high atomic number ($Z = 83$) compared to clinically approved iodine-based ($Z = 53$) or barium ($Z=56$) contrast agents, thus enhancing X-ray opacity (Lusic and Grinstaff, *Chemical Reviews*, Vol. 113, 2013, pp. 1641-1666). The potential
25 of metallic Bi NPs as XCA was demonstrated in three recent studies (Brown et al., *Chemistry of Materials*, Vol. 26, 2014, pp. 2266-2274; Wei et al., *ACS Applied Materials and Interfaces*, Vol. 8, 2016, pp. 12720-12726; Swy et al., *Nanoscale*, Vol. 6, 2014, pp. 13104-13112). Moreover, Bi NPs can be incorporated in different medical devices and implantable medical materials to give them an X-ray radioopacity, especially in polymers
30 or plastics to provide radiopaque polymeric materials used as medical implants or inserts.

Bi NPs have also been described as having bactericide, fungicide, antiparasitic, antibiofilm, antibiofouling, antiviral properties.

In view of the above, Bi NPs have several applications and especially promising medical potencies. Especially, Bi NPs may be compared in terms of medical potencies to well-studied Au NPs (Yang et al., Chemical Reviews, Vol. 115, 2015, pp. 10410-10488).
5 Nevertheless, the manufacturing of Au NPs is definitely easier and enables a better size and morphological control with comparison with processes of manufacturing of Bi NPs currently described in literature. Therefore, Au NPs currently remain widely preferred even if gold is about 2000 times more expensive than bismuth.

10 There is thus a need for an improved Bi NPs manufacturing process in order to enable a wider use of Bi NPs, especially in the medicinal field.

Among the nanostructures that may exist for Bi NPs, monodisperse spherical NPs are of particular interest in reasons of their unique optical, catalytic, chemical and biological properties.

15 While numerous studies are published on the synthesis and characterization of bismuth oxide or bismuth sulphide nanoparticles, only a few works are dealing with the synthesis and applications of spherical Bi NPs. Two main strategies are described in the literature to synthesize Bi NPs.

The first strategy uses a top-down approach to reduce the size of a bulk bismuth material
20 at a nanometer scale by using physical procedures (Zhao et al., Material Letters, Vol. 58, 2004, pp. 790-793; Wang et al., Nano Letters, Vol. 4, No. 10, 2004, pp. 2047-2050). Nevertheless, the top-down approach requires the use of an elevated temperature which is in contradiction with green chemistry principles and may cause process safety concern in an industrial production context.

25 The second strategy uses a bottom-up approach to obtain Bi NPs by combination between bismuth metallic (0) and a capping agent. In this strategy, bismuth (0) is obtained by a reduction from bismuth (III) or more rarely bismuth (V). Several reductive conditions are described with a wide variety of reductants, solvents, bismuth sources and capping agents.

Growth and stabilization of Bi (0) nuclei are dependent on capping agents, solvent, temperature, concentration of the reactants and stirring rates.

- A first bottom-up approach involves thermal decomposition of bismuth salts solubilized in high boiling organic solvents in presence of capping agents. Such thermolytic process with very high temperature does not fulfill criteria of green nanochemistry. Moreover, sensitive bismuth precursors with undocumented toxicity and high-boiling point solvents difficult to remove need to be used in these process. Furthermore, thermal decomposition needs to be carried out under anaerobic and anhydrous conditions, and temperature and reaction time have to be finely controlled, rendering scale-up difficult.
- 10 A second bottom-up approach involves polyol solvents, using bismuth precursors which can be in oxidation state (III) or more rarely in oxidation state (V). This approach provides Bi NPs with well-defined shapes and controlled sizes. Polyol solvents enable to dissolve inorganic compounds, offer a wide operating temperature range and may also serve as reducing agents. Polyols can also act as stabilizers to control particle growth and to prevent particle aggregation and/or agglomeration. An example of polyol process is provided by Wu (Wu et al., Journal of Alloys and Compounds, Vol. 498, 2010, pp. L8-L11), using bismuth (III) citrate as precursor, urea as stabilizing agent and diethylene glycol used as polyol solvent and as reducing agent, carried out under fast microwave irradiation, and provides large quantities of Bi NPs with diameters of most spherical Bi NPs ranging from 400 nm to 700 nm. Urea and diethylene glycol form a coating layer around the bismuth core stabilizing the nanoparticles. Even if this efficient polyols process has some interest in green nanochemistry, it is not adapted to provide smaller monodisperse Bi NPs than those described.

- A third bottom-up approach involves reduction processes of bismuth (III) precursor in organic solvents in presence of a reducing agent. The synthesis of metallic Bi NPs by reduction in organic solvent allows to obtain a size range of 2-74 nm depending on experimental conditions as solvent, reductive agent and temperature. In particular, Bi NPs have been prepared in DMSO (Velasco-Arias et al., The Journal of Physical Chemistry, Vol. 116, 2012, pp. 14717-14727; Hernandez-Delgadillo et al., International Journal of Nanomedicine, Vol. 7, 2012, pp. 2109-2113), however these Bi NPs degrade over time

by dissolution. The main drawback of this method in terms of green nanochemistry is the use of strong and potentially toxic reducing agent (such as borohydride) in stoichiometric amounts. These protocols are carried out anaerobically and potentially toxic and expensive organic solvents are used. It is evidenced in the experimental part below (example 1b) that the nanoparticles of Hernandez-Delgadillo et al. are stabilized by DMSO that form a coating layer on the bismuth core. Luo et al. also disclose the manufacturing of Bi NPs by a reduction processes of bismuth (III) precursor (bismuth (III) nitrate) in organic solvents (DMF) in presence of a reducing agent (sodium borohydride) and in presence of polyvinylpyrrolidone as stabilizing agent (J Mater. Sci.: Mater. Med., 2012, 23, 2563-2573). This synthesis presents the same drawbacks.

A fourth bottom-up approach involves reduction processes in water. Aqueous preparations of Bi NPs are clearly attractive because water is a green solvent, easily removed by filtration contrary to organic solvent and suitable for medical applications. Nevertheless, several difficulties are associated with water as solvent: (1) it usually requires the use of strong and toxic reducing agents; (2) most commonly used bismuth salts are insoluble in aqueous medium; and (3) prepared Bi NPs can be easily hydrolyzed or oxidized in water.

Using additives such as EDTA (Fu et al., Crystal Growth & Design, Vol. 5, No. 4, 2005, pp. 1379-1385), tartaric acid (Ma et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects, Vol. 395, 2012, pp. 276-283) or ammonium bismuth citrate (Xia et al., Industrial and engineering chemistry research, Vol. 53, 2014, pp. 10576-10582) enables overcoming the lack of solubility of bismuth precursors in water.

The selection of capping agents should be careful in order to avoid Bi NPs oxidation in water. In literature, oleate is used as capping agent to form a stabilizing barrier of Bi NPs (Ma et al. 2012). Nevertheless, this hydrophobic agent prevents colloidal dispersion of resulting Bi NPs in water. In these aqueous synthesis, hydrophilic polymeric capping agents were also used to obtain stable Bi NPs dispersion in water such as polymeric starch (Xia et al. 2014), 75 kDa dextran or PVP (Fu et al., 2005). Thus, in the aqueous preparations of Bi NPs of the prior art, only polymeric and/or hydrophobic coating agents were used.

- WO2012/170569 also discloses the manufacturing of Bi NPs in water using bismuth nitrate as bismuth precursor and boranes or borohydride as reducing agent, in presence of dextran as stabilizing coating. In WO2012/170569, the formation of the Bi NPs is based on a pH-dependent dissolution mechanism of solid bismuth hydroxide in equilibrium with aqueous bismuth (III) and hydroxide ions. In other words, in WO2012/170569, the pH is settled so as to obtain a partial solubilizing of the bismuth precursor in order to enable a progressive formation of the NPs. This process presents the drawback of not being suitable of continuous flow manufacturing due to the presence of unsolubilized bismuth precursor. Moreover, it requires the use of strong and toxic reducing agents.
- 10 CN10466578 also discloses the manufacturing of Bi NPs in water, using bismuth nitrate as bismuth precursor and glucose as reducing agent, in presence of alkali metal hydroxide such as sodium hydroxide. CN10466578 states that prepared Bi NPs remain stable for 30 days, on the basis of transmission electron microscopy (TEM) measurements. TEM measurements enable to determine the size of the metallic core of nanoparticles. By definition, the size of the metallic core of each nanoparticle does not change upon aggregation of the nanoparticles. Thus, assessing the colloidal stability of nanoparticles is not possible by TEM. On the contrary, using dynamic light scattering (DLS) measurements enables determining if aggregation occurs in a nanoparticle suspension. Therefore, the Applicant reproduced example 1 of CN10466578 (see experimental part –
- 20 Example 1a) and evidenced that (1) the bismuth precursor used in this process is insoluble in the aqueous medium, would lead to problems of reproducibility and of scale up especially in a continuous flow process; (2) the Bi NPs obtained by this process are not colloidally stable, i.e. in term of aggregation and/or agglomeration, as shown by DLS measurements; and (3) obtained Bi NPs are of wide size.
- 25 Conventionally, nanoparticles are produced with batch processes. One of the drawback of batch processes is the lack of suitable control over mixing and mass and heat transfer, which creates particles with wide size distribution, irreproducibility of size, and morphology. Besides, it is difficult to obtain the same result when scaling up a batch process, that is, to achieve process reliability. For these reasons in addition to productivity
- 30 aspects, it is particularly relevant to implement processes in continuous flow.

Therefore, even if various synthesis methods have been reported for Bi NPs, there is still a need for a simple, low-cost and rapid approach, easy to scale-up. Especially, there is a need for a method for manufacturing Bi NPs which:

- 5 – is conducted in green conditions, for example using water as solvent, a biocompatible reducing agent and/or a water-soluble bismuth precursor;
- may easily be scaled-up, and especially may be adapted to continuous flow;
- provides monodisperse Bi NPs, and preferably Bi NPs of small size so that the Bi NPs are more easily excreted *in vivo* and are able to diffuse in biological tissues;
- 10 – provides stabilized Bi NPs with regards to (1) agglomeration and/or aggregation, i.e. colloidal stability in solution, and (2) to degradation by dissolution;
- provides Bi NPs stable with regards to sterilization process;
- provides functionalizable Bi NPs either by hydrophilic, hydrophobic, 15 fluorophilic or bioactive groups, which may thus be formulated in aqueous, lipophilic or fluorophilic media.

The present invention provides a process for manufacturing Bi NPs meeting above specifications. The process of the invention is performed in aqueous medium, starting from a water-soluble bismuth precursor and optionally in presence of a coating and 20 leading to stabilized Bi NPs. The process of the invention is adapted to a continuous flow process. It enables providing stable Bi NPs which may be functionalized and sterilized. The process of the invention also presents the advantage to enable a reproducible control of the nanoparticles size.

25 SUMMARY

This invention thus relates to a process for manufacturing biocompatible bismuth (0) nanoparticles comprising a core comprising bismuth (0), surrounded by at least one coating layer adsorbed thereon, said process being performed in aqueous solution, and characterized in that it comprises the steps of:

a) preparing an aqueous solution with a pH greater than 12, said aqueous solution comprising:

- a water-soluble bismuth precursor of formula Bi(III)-L totally dissolved in the solution, wherein L is a stabilizing group selected from polycarboxylic acid and thiocarboxylic acid;
- a water-soluble reducing agent selected from monosaccharides;
- optionally one or more coating agent of formula X-L'; and
- water;

wherein:

X is a cation, preferably a pharmaceutically acceptable cation, more preferably a cation selected from hydrogen cation sodium and ammonium; and

L' is a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, and biotargeting derivatives thereof of formula L¹-A-L², wherein:

L¹ is selected from polycarboxylic acid, thiocarboxylic acid and thioalcohol;

L² is a biotargeting group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol and zwitterionic groups; and
- a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances; and

A is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene;

polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;

- 10 b) heating the aqueous solution obtained in step a); and
- c) recovering the bismuth (0) nanoparticles from the aqueous solution obtained in step b).

In one embodiment, in the process of the invention, the polycarboxylic acid is citric acid; thiocarboxylic acid is dimercaptosuccinic acid, glutathione or lipoic acid; and thioalcohol
15 is dimercaptopropanol or 2-mercaptoethanol. In one embodiment, in the process of the invention, the monosaccharide water-soluble reducing agent is glucose.

In one embodiment, the process further comprises a step of sterilization, preferably a step of sterilization under heating.

In one embodiment, the recovering of step c) is made by filtration, preferably by
20 ultrafiltration.

In one embodiment, the process of the invention comprises an additional step of coating-exchange during which the bismuth (0) nanoparticles of step c) are put in presence of a different coating agent of formula X''-L'', wherein:

X'' is a cation, preferably a pharmaceutically acceptable cation, more preferably
25 a cation selected from hydrogen cation, sodium and ammonium; and
L'' is a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic

acid, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$, wherein:

$L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine, and

$L^{2''}$ is a group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
- a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and
- a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, selected from saturated or unsaturated C_3F_7 to $C_{18}F_{37}$ perfluorinated chains and partially fluorinated alkyl chains; and

A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups

selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-.

In one embodiment, the process of the invention comprises an additional step of silanization; optionally followed by a step of functionalization of silica by one or more organohydroxysilane group functionalized by:

- 10 - a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
- a bioactive group, selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or
- 15 trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from hydroxysilane groups such as alkyl hydroxysilane, metacrylate
- 20 hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octohydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane,
- 25 octadecylhydroxysilane or methacryloxypropylhydroxysilane; and
- a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, selected from perfluorinated organohydroxysilane groups and partially fluorinated alkyhydroxysilane groups such as fluoromethylhydroxysilane 2-
- 30 fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-

nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-
 nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxysilane,
 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-
 pentafluorobutylhydroxysilane.

5 In one embodiment, the process of the invention is carried out in continuous flow.

The present invention also relates to bismuth (0) nanoparticles obtained by the process of the invention.

The present invention further relates bismuth (0) nanoparticle comprising:

- a core comprising bismuth (0);
- 10 - surrounded by at least one coating layer adsorbed on the core, comprising:
 - (1) at least one stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1'}-A''-L^{2'}$, wherein:
 - 15 $L^{1'}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid and cyclodextrine; and
 - $L^{2''}$ is a group selected from:
 - a hydrophilic group having an effect on biodistribution or on macrophage capture selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
 - 20 - a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
 - 25 - a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and

- a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, selected from saturated or unsaturated C₃F₇ to C₁₈F₃₇ perfluorinated chains and partially fluorinated alkyl chains; and
- A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;
- and/or
- (2) at least one organohydroxysilane group functionalized by a group selected from:
- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
 - a bioactive group, selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;

- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from hydroxysilane groups such as alkyl hydroxysilane, metacrylate hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octohydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane, octodecylhydroxysilane or methacryloxypropylsilane; and
- a fluorophilic group capable of solubilizing Bi NPs in an fluorous phase, selected from perfluorinated organohydroxysilane groups or partially fluorinated alkylhydroxysilane groups such as fluoromethylhydroxyysilane 2-fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxyysilane, 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-pentafluorobutylhydroxysilane;

and

the nanoparticle comprises from 1 to 40 stabilizing groups per nm²,

the nanoparticle size is smaller than 40 nm measured by Transmission Electron Microscopy (TEM);

the ratio of the nanoparticle hydrodynamic diameter measured by dynamic light scattering (DLS) in number to the nanoparticle size measured by TEM is ranging from 1 to 45, and

the nanoparticle does not contain boron derivatives.

The invention also relates to a composition comprising bismuth (0) nanoparticles according to the invention and a carrier.

The invention also provides a delivery device comprising a container and an injection
5 device, the said container containing a composition according to the invention.

The invention also relates to bismuth (0) nanoparticles according to the invention for use as medicament; preferably as radiosensitizer agent in radiotherapy or in photothermal therapy, or as biocide agent.

It is also provided the non-therapeutic use of bismuth (0) nanoparticles according to the
10 invention as biocide agent; preferably as bactericide, antimicrobial, antibacterial, antimycotical, fungicide, antiparasitic, antibiofilm, antibiofouling or antiviral agent.

The invention further relates the use of bismuth (0) nanoparticles according to the invention as radioopacifying agent, preferably as X-ray contrast agent for medical imaging or as radioopacifying agent in medical devices.
15

DEFINITIONS

In the present invention, the following terms have the following meanings:

The term “**administration**”, or a variant thereof (e.g. “**administering**”), means providing the active agent or active ingredient, alone or as part of a pharmaceutically acceptable
20 composition, to the patient in whom/which the condition, symptom, or disease is to be treated or prevented.

The term “**alkenyl**” refers to an unsaturated hydrocarbyl group, which may be linear or branched, wherein the unsaturation arises from the presence of one or more carbon-carbon double bonds. Suitable alkenyl groups comprise between 2 and 6 carbon atoms.
25 Non-limiting examples of alkenyl groups are ethenyl, propenyl, butenyl, pentenyl and hexenyl.

The term “**alkenylene**” refers to an alkenyl group as herein defined having two single bonds as points of attachment to other groups.

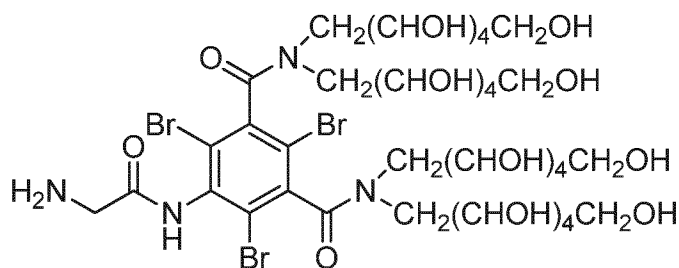
The term “**alkoxyalkylene**” refers a group –alkyl–O–alkyl–, wherein alkyl is as herein defined.

- 5 The term “**alkyl**”, by itself or as part of another substituent, refers to a hydrocarbyl radical of Formula C_nH_{2n+1} , wherein n is a number greater than or equal to 1. Generally, alkyl groups of this invention comprise from 1 to 6 carbon atoms. Alkyl groups may be linear or branched. Examples of alkyl groups are methyl, ethyl, n-propyl, i-propyl, butyl and its isomers (e.g. n-butyl, i-butyl and t-butyl); pentyl and its isomers, hexyl and its isomers.
- 10 The term “**alkylene**” refers to an alkyl group as herein defined having two single bonds as points of attachment to other groups. The term alkylene includes methylene, ethylene, methylenemethylene, propylene, ethylethylene and 1,2-dimethylethylene.

- The term “**alkynyl**” refers to an unsaturated hydrocarbyl groups, which may be linear or branched, wherein the unsaturation arises from the presence of one or more carbon-carbon triple bonds. Alkynyl groups typically, and preferably, have the same number of carbon atoms as described above in relation to alkenyl groups. Non limiting examples of
- 15 alkynyl groups are ethynyl, 2-propynyl, 2-butylnyl, 3-butylnyl, 2-pentylnyl and its isomers, 2-hexynyl and its isomers-and the like.

- The term “**alkynylene**” refers to an alkynyl group as herein defined having two single
- 20 bonds as points of attachment to other groups.

- The term “**aminoalcohol**” refers to chemical groups comprising at least one amine group and at least one hydroxyl group. As simple example of aminoalcohol is ethanolamine. Among aminoalcohols suitable as hydrophilic group having an effect on biodistribution or on macrophage capture there is for example aminoalcohols of formula R^1-NH-R^2
- 25 wherein R^1 and R^2 are the same or different and represent an aliphatic hydrocarbon chain comprising from 2 to 6 carbon atoms, substituted preferably by 6 to 10 hydroxyl groups or by 4 to 8 hydroxyl groups in the case wherein R^1 and/or R^2 is interrupted, especially when R_1 represents a group $-(CH_2)-(CHOH)_4-CH_2OH$ or $-(CH_2)-CHOH-CH_2OH$ and R^2 is a group $-CH_2-(CHOH)_4-CH_2OH$, and especially the group:



The term “**aminosaccharide**” refers to a saccharide in which a hydroxyl group has been replaced with an amine group. An example of aminosaccharide is glucosamine.

The term “**antibody**” refers to monoclonal antibodies (mAb), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), hybrid or chimeric antibodies and antibody fragments, so long as they exhibit the desired biological activity. An “antibody fragment” comprises a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870; Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules, especially single-chain variable fragment (scFv); and multispecific antibodies formed from antibody fragments.

The term “**aqueous solution**” refers to solution wherein the solvent is water.

The term “**aryl**” refers to a polyunsaturated, aromatic hydrocarbyl group having a single ring (i.e. phenyl) or multiple aromatic rings fused together (e.g. naphthyl), or linked covalently, typically containing 5 to 12 atoms; preferably 6 to 10, wherein at least one ring is aromatic. The aromatic ring may optionally include one to two additional rings (either cycloalkyl, heterocyclyl or heteroaryl) fused thereto. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated herein. Non-limiting examples of aryl comprise phenyl, biphenyl, biphenylenyl, 5- or 6-tetralinyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-azulenyl, naphthalen-1- or -2-yl, A-, 5-, 6 or 7-indenyl, 1- 2-, 3-, 4- or 5-acenaphtylenyl, 3-, 4- or 5-acenaphtenyl, 1-, 2-, 3-, 4- or 10-phenanthryl, 1- or 2- pentalenyl, 4- or 5-indanyl, 5-, 6-, 7- or 8-tetrahydronaphthyl, 1,2,3,4-tetrahydronaphthyl, 1,4-dihydronaphthyl, 1-, 2-, 3-, 4- or 5-pyrenyl.

The term “**bioactive group**” refers to a molecule being able to recognize a predefined biological target. Preferably, “bioactive group” refers to biomolecules or organic compounds. “Biomolecules” include antibodies, peptides, proteins, polysaccharide, fatty acid, hapten, and polyamine; selected to bind biological targets but also molecular
5 scaffold able to produce a signal in medical imaging (MRI, TEP, SPECT, fluorescence).

In one embodiment, preferred bioactive group are selected from glycoproteins, lectins, biotin, vitamins, pteroid or aminopteroic acid derivatives, folic acid or antifolic acid derivatives, antibodies or antibody fragments, peptides and derivatives thereof, mono- or polysaccharides, saccharides, avidin, inhibitors or substrates of receptors (membrane or
10 nucleic receptors), phospholipids and derivatives thereof, steroids and analogs thereof, oligonucleotides, ribonucleic acid sequences, deoxyribonucleic acid sequences, hormones or substances analogs to hormones, amino acids, organic molecules having a pharmacological activity, pharmacophores, proteins eventually recombinant or mutated.

In a preferred embodiment, the bioactive group is selected from (references are examples only and non-limitative):
15

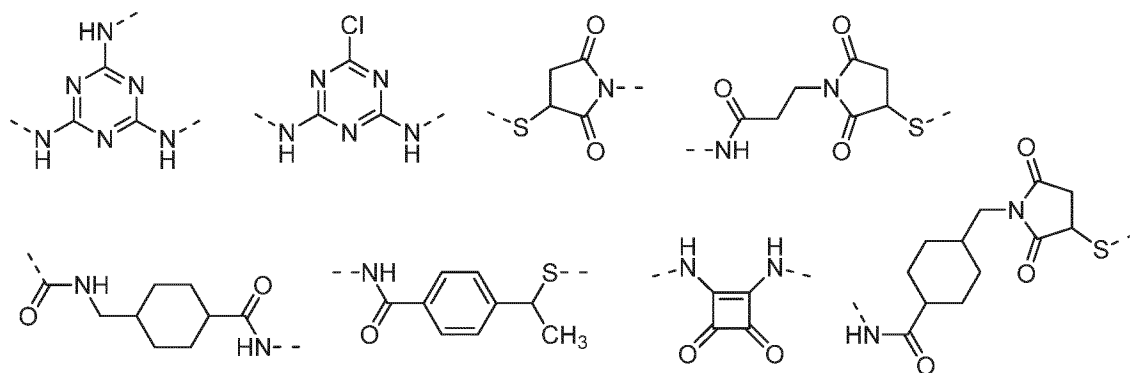
1) bioactive groups targeting VEGF and angiopoietin receptors (described in WO 01/97850), polymers such as polyhistidine (US 6,372,194), polypeptides targeting fibrin (WO 2001/9188), peptides targeting integrins (WO 01/77145, WO 02 26776 for alpha v beta 3, WO 02/081497, for example RGDWXX), pseudopeptides and peptides
20 targeting MMP metalloproteases (WO 03/062198, WO 01/60416), peptides targeting for example KDR/Flk-1 receptor among which R-X-K-X-H and R-X-K-X-H, or Tie-1 and 2 receptors (WO 99/40947 for example), sialyl Lewis glycosides (WO 02062810 and Müller et al, Eur. J. Org. Chem, 2002,3966-3973), antioxidants such as ascorbic acid (WO 02/40060), biovectors targeting tuftsin (for example US 6,524,554),
25 biovectors targeting protein G receptors GPCR especially cholecystokinin (WO 02/094873), associations between integrin antagonist and guanidine mimic (US 6 489 333), quinolones targeting alpha v beta 3 or 5 (US 6,511,648), benzodiazepines and analogs thereof targeting integrins (US A 2002/0106325, WO01/97861), imidazoles analogs thereof (WO 01/98294), RGD peptides (WO 01/10450), antibodies or antibody
30 fragments (FGF, TGFb, GV39, GV97, ELAM, VCAM, inducible by TNF or IL (US

- 6,261,535), targeting molecules modified by interaction with the target (US 5,707,605), targeting agents amyloids deposits (WO 02/28441), cathepsins peptides (WO 021056670), mitoxantrone or quinone (US 6,410,695), polypeptides targeting epithelial cells (US 6,391,280), inhibitors of cysteines proteases (WO 99/54317), biovectors disclosed in: US 6,491,893 (GCSF), US 2002/0128553, WO 02/054088, WO 02132292, WO 02/38546, WO20036059, US 6,534,038, WO 0177102, EP 1 121 377, Pharmacological Reviews (52, n°2, 179 ; growth factors PDGF, EGF, FGF...), Topics in Current Chemistry (222, W.Krause, Springer), Bioorganic & Medicinal Chemistry (11, 2003, 1319-1341 ; tetrahydrobenzazepinone derivatives targeting α v β 3),
- 2) angiogenesis inhibitors, especially those tested in clinical trials or already commercialized, such as for example:
- angiogenesis inhibitors implying FGFR or VEGFR receptors such as SUI 01, SU5416, SU6668, ZD4190, PTK787, ZK225846, azacyclyl compounds (WO 00244156, WO 02059110);
 - angiogenesis inhibitors implying MMP such as BB25-16 (marimastat), AG3340 (prinomastat), solimastat, BAY12-9566, BMS275291, metastat, neovastat;
 - angiogenesis inhibitors implying integrins such as SM256, SG545, adhesion molecules blocking EC-ECM (such as EMD 121-974 or vitaxine);
 - drugs having indirect antiangiogenic mechanism of action such as carboxiamidotriazole, TNP470, squalamine, ZD0101;
 - inhibitors disclosed in WO 99/40947, monoclonal antibodies selective for the binding to KDR receptor, somatostatin analogs (WO 94/00489), selectin binding peptides (WO 94/05269), growth factors (VEGF, EGF, PDGF, TNF, MCSF, interleukins); biovectors targeting VEGF described in Nuclear Medicine Communications, 1999, 20;
 - inhibiting peptides of WO 02/066512;
- 3) biovectors able to target the following receptors: CD36, EPAS-1, ARNT, NHE3, Tie-1, 1/KDR, Flt-1, Tek, neuropiline-1, endoglin, pleiotropin, endosialin, AxL, α 1Pi, α 2ssl, α 4P1, α 5pl, eph B4 (ephrin), laminin A receptor, neutrophilin 65 receptor, leptin OB-RP receptor, chemokine CXCR-4 receptor (and other receptors cited in WO99/40947), LHRH, bombesin/GRP, gastrin receptor, VIP, CCK;

- 4) tyrosine kinase inhibitors biovectors;
- 5) inhibitors of GPIIb/IIIa receptor selected from: (1) fab fragment of a monoclonal antibody of GPIIb/IIIa receptor, Abciximab, (2) small peptidic and peptidomimetic molecules such as eptifibatide or tirofiban;
- 5 6) antagonist peptides of fibrinogen receptor (EP 425 212), peptides which are ligands of IIb/IIIa receptors, fibrinogen ligands, thrombin ligands, peptides able to target atheroma plaque, platelets, fibrin, hirudin-based peptides, guanine-based derivatives targeting IIb/IIIa receptor;
- 7) other biovectors or biologically active fragments of biovectors known by one skilled
10 in the art as drugs, with anti-thrombotic, anti-platelet aggregation, anti-atherosclerotic, anti-restenotic, anticoagulant action;
- 8) other biovectors or biologically active fragments of biovectors targeting avj33, described in association with DOTAs in US 6,537,520, selected from: mitomycin, tretinoin, ribomustine, gemcitabine, vincristine, etoposide, cladribine, mitobronitol,
15 methotrexate, doxorubicin, carboquone, pentostatin, nitracrin, zinostatin, cetorelix, letrozole, raltitrexed, daunorubicin, fadrozole, fotemustine, thymalfasin, sobuzoxane, nedaplatin, cytarabine, bicalutamide, vinorelbine, vesnarinone, aminoglutethimide, amsacrine, proglumide, elliptinium acetate, ketanserin, doxifluridine, andretinate , isotretinine, streptozocin, niur, razoxane, sizofilan, carboplatin, mitolactol, tegafur,
20 ifosfamide, prednimustinemustine, vindesine, flutamide, drogenil, butocin, carmof, picibanil, levamisole, teniposide, improsulfan, enocitabine, lisuride, oxymetholone, tamoxifen, progesterone, mepitiostane, epitiostanol, formestane, interferon-alpha, interferon-2-alpha, interferon-beta, interferon-gamma, colony stimulating factor-1, colony stimulating factor-2, denileukin diftitox, interleukin-2, leutinizing hormone
25 releasing factor;
- 9) some biovectors targeting specific types of cancers, for example peptides targeting ST receptor associated to colorectal cancer, or the tachykinin receptor;
- 10) biovectors using phosphine-type compounds;
- 11) biovectors targeting P-selectin, E-selectin; for example, the 8 amino acid peptide
30 described by Morikawa et al, 1996, 951, as well as various sugars;
- 12) annexin V or biovectors targeting apoptotic processes;

- 13) any peptide obtained by targeting technologies such as phage display, optionally modified by unnatural amino acids, for example peptides from phage display libraries: RGD, NGR, CRRETAWAC, KGD, RGD-4C, XXXY * XXX, RPLPP, APPLPPR;
- 14) other known peptide biovectors targeting atheroma plaques, such as those cited in
5 WO 20031014145;
- 15) vitamins;
- 16) hormonal receptor ligands including hormones and steroids;
- 17) biovectors targeting opioid receptors;
- 18) LB4 and VnR antagonists;
- 10 19) nitriimidazole compounds and benzylguanidines;
- 20) biovectors recalled in Topics in Current Chemistry, vol.222, 260-274, Fundamentals of Receptor-based Diagnostics Metallopharmaceuticals, including:
- biovectors for targeting peptide receptors overexpressed in tumors (LHRH receptors, bombesin / GRP receptors, VIP receptors, CCK receptors, tachykinin receptor
15 receptors, for example), in particular somatostatin or bombesin analogues, peptides derived from octreotide possibly glycosylated, VIIP peptides, alpha-MSH, CCK-B peptides;
 - peptides chosen from: cyclic RGD peptides, fibrin-alpha chain, CSVTCR, tuftsin, fMLF, YIGSR (receptor: laminin);
- 20 21) oligosaccharides, polysaccharides and derivatives of oses, derivatives targeting Glut receptors (ose receptors);
- 22) biovectors used for smart-type products;
- 23) markers of myocardial viability (tetrafosmin and hexakis2methoxy-2-methylpropylisonitrile);
- 25 24) tracers of the metabolism of sugars and fats;
- 25) neurotransmitter receptor ligands (D, 5HT, Ach, GABA, NA receptors);
- 26) oligonucleotides;
- 27) tissue factor;
- 28) biovectors described in WO 03120701, in particular the PK11195 ligand of peripheral
30 benzodiazepines receptor;

- 29) fibrin-binding peptides, in particular the peptide sequences described in WO 03/11115;
- 30) amyloid plaque aggregation inhibitors described in WO 02/085903;
- 31) compounds targeting Alzheimer's disease, in particular compounds comprising
 5 benzothiazole, benzofurans, styrylbenzoxazoles/thiazoles/imidazoles/quinoline or
 styrylpyridines backbones;
- 32) antimicrobial peptides (Reihardt Int J Molecular Sciences 2016, 17,701) or
 antimicrobial metallo peptides (Alexander ACS Chem Biol 2018, 13, 844-853);
- 33) small bioactive peptides (Hamley Chem. Rev., 2017, 117 (24), pp 14015–14041).
- 10 The bioactive groups, especially the antibodies and the pharmacophores, may be
 optionally functionalized so as to be able to react with the other part of the stabilizing
 group and form a covalent bond therewith, such as for example the following bonds:
 -CONH-, -COO-, -NHCO-, -OCO-, -NH-CS-NH-, -C-S-, -N-NHCO-, -CO-NH-N-,
 -CH₂-NH-, -N-CH₂-, -N-CS-N-, -CO-CH₂-S-, -N-CO-CH₂-S-, -NCO-CH₂-CH₂-S-,
 15 -CH=NH-NH-, -NH-NH=CH-, -CH=NO-, -ON=CH- or corresponding to the following
 formulae:



- The term “**biocide agent**” refers to a substance able to destroy, deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means. By
 20 “biocide agent” is thus referred to a compound that has bactericide, antimicrobial,
 antibacterial, antimycotical, fungicide, antiparasitic, antibiofilm, antibiofouling, antiviral
 properties. A biocide agent can be a pesticide, including fungicides, herbicides,
 insecticides, algicides, molluscicides, miticides and rodenticides; or an antimicrobial,

including germicides, antibiotics, antibacterials, antivirals, antifungals, antiprotozoals and antiparasites.

The term “**biocompatible**” refers to a material eliciting little or no immune response in a given organism, or is able to integrate with a particular cell type or tissue.

- 5 The term “**biotargeting group**” refers to a group that targets a biological target or a biological mechanism.

The term “**bismuth precursor**” refers to a salt of bismuth, preferably a salt of bismuth (III).

- 10 The term “**coating agent**” refers to a group allowing to manage the stability and solubility of the Bi NPs in aqueous, hydrophobic (or oily) or fluoruous phase. The Bi Nps surface can be tuned via the selection of one or more coating agent. In a preferred embodiment, the coating agent may be hydrophilic, hydrophobic, amphiphilic or fluorophilic.

The term “**coating exchange**” refers to a process where the coating of the Bi NPs is exchanged with another coating agent that has a greater affinity for the Bi NPs surface.

- 15 The term “**coating layer**” refers to a layer which is present at the surface of the metallic core of the Bi NPs, and comprising at least one optionally functionalized stabilizing group and/or optionally functionalized silica. The coating layer may be continuous or discontinuous. The coating layer may comprise one or more optionally functionalized stabilizing group and/or optionally functionalized silica. In the present invention, the
20 coating layer is adsorbed on the bismuth (0) metallic core.

The term “**continuous flow**” refers to an approach to discrete manufacturing that contrasts with batch production. The goal is an optimally balanced production line with little waste, highest productivity, the lowest possible cost, on-time and defect-free production.

- 25 The term “**fluorophilic group**” refers to a group having a chemical affinity for fluorocarbons, enabling it to dissolve more readily in fluoruous phase.

The term “**fluoruous phase**” refers to a solvent comprising perfluorinated or partially fluorinated groups, such as for example linear or branched, cyclic or polycyclic, saturated

or unsaturated fluorocarbon oils; cyclic tertiary fluorinated amines; fluorinated esters or thioesters; halofluorocarbons; and derivatives thereof (see for example FR2 980 365; "Fluorous solvents and related systems", By Anon. RSC Green Chemistry Series (2013), 20 (Alternative Solvents for Green Chemistry), pp. 210-241; "Fluorous solvents", Ryu, 5 Ilhyong et al., Edited By: Mikami, Koichi. Green Reaction Media in Organic Synthesis (2005), pp. 59-124). Preferably, at least 15% of the hydrogen atoms of the corresponding hydrocarbon oil are replaced by fluoride atoms. When 100% of the hydrogen atoms are replaced by fluoride atoms, it corresponds to the "**perfluorinated**" oil. Typically, these fluorinated oils are chains of 2 to 16 atoms, perfluoroalkanes, 10 bis(perfluoroalkyle)alkenes, perfluorethers, perfluoroamines, perfluoroalkyle bromides, perfluoroalkyle chloride or corresponding partially fluorinated compounds.

The term "**heteroaryl**" refers to 5 to 12 carbon-atom aromatic rings or ring systems containing 1 to 2 rings which are fused together or linked covalently, typically containing each 5 to 6 atoms; at least one of which is aromatic in which one or more carbon atoms 15 in one or more of these rings can be replaced by oxygen, nitrogen or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. Such rings may be fused to an aryl, cycloalkyl, heteroaryl or heterocyclyl ring. Non-limiting examples of such heteroaryl include: pyrrolyl, furanyl, thiophenyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, 20 thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, tetrazolyl, oxatriazolyl, thiatriazolyl, pyridinyl, pyrimidyl, pyrazinyl, pyridazinyl, oxazinyl, dioxinyl, thiazinyl, triazinyl, imidazo[2,1-b][1,3]thiazolyl, thieno[3,2-b]furanyl, thieno[3,2-b]thiophenyl, thieno[2,3-d][1,3]thiazolyl, thieno[2,3-d]imidazolyl, tetrazolo[1,5-a]pyridinyl, indolyl, indolizinyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, 25 isobenzothiophenyl, indazolyl, benzimidazolyl, 1,3-benzoxazolyl, 1,2-benzisoxazolyl, 2,1-benzisoxazolyl, 1,3-benzothiazolyl, 1,2-benzoisothiazolyl, 2,1-benzoisothiazolyl, benzotriazolyl, 1,2,3-benzoxadiazolyl, 2,1,3-benzoxadiazolyl, 1,2,3-benzothiadiazolyl, 2,1,3-benzothiadiazolyl, thienopyridinyl, purinyl, imidazo[1,2-a]pyridinyl, 6-oxo-pyridazin-1(6H)-yl, 2-oxopyridin-1 (2H)-yl, 6-oxo-pyridazin-1(6H)-yl, 2-oxopyridin- 30 1(2H)-yl, 1,3-benzodioxolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, quinoxalinyl.

The term “**hydrodynamic diameter**” refers to the apparent size of the dynamic hydrated/solvated particle, including a hydration layer surrounding the particle. It is measured by Dynamic Light Scattering (DLS), in number, in intensity or in volume; preferably in number. The hydrodynamic diameter is calculated from the diffusional properties of the particle.

The term “**hydrophilic group**” refers to a group that is typically charge-polarized and capable of hydrogen bonding, enabling it to dissolve more readily in water than in oil or other solvents. Examples of hydrophilic groups are aminoalcohol, polyethylene glycol and zwitterionic groups.

10 The term “**hydrophilic group having an effect on biodistribution or on macrophage capture**” refers to a hydrophilic group having an effect on the repartition in biological tissues of the nanoparticles and on its uptake by macrophages. In a preferred embodiment, the hydrophilic grouping an effect on biodistribution or on macrophage capture is selected from aminoalcohol, polyethylene glycol (PEG) or zwitterionic groups.

15 The term “**hydrophobic group**” or “**lipophilic group**” refers to a group that is typically non-polar, enabling to dissolve more readily in oil (i.e. oily phase) or other hydrophobic solvents than in water.

The term “**monosaccharide**” refers to polyhydroxy aldehydes or polyhydroxy ketones, comprising at least carbon atoms, and which are not hydrolysable. Non limitative examples of monosaccharides are trioses (glyceraldehyde, dihydroxyacetone); tetroses (erythrose, threose, erythrulose); pentoses (desoxyribose, ribose, arabinose, xylose, lyxose, ribulose, xylulose); hexoses (allose, altrose, galactose, glucose, gulose, idose, mannose, talose, fructose, psicose, sorbose, tagatose); desoxy-hexoses (fucose, rhamnose); heptoses (sedoheptulose, mannoheptulose); nonoses (neuraminic acid or sialic acid).

25 The term “**nucleic acid**” refers to a polymer of nucleotides covalently linked by phosphodiester bonds, such as deoxyribonucleic acids (DNA) or ribonucleic acids (RNA), in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner

30

similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated.

- 5 The term “**oily phase**” or “**oil**” refers to a solvent that is nonpolar and does not mix with water. Oils include organic oils and mineral oils. Examples of oils are medium chain triglycerides (such as Mygliol® oils) or ethyl esters of iodized fatty acids of poppy-seed oil (such as LIPIODOL®).

The term “**organoalkylsilane**” refers to a group alkyl–SiO–alkyl, wherein alkyl is as
10 herein defined.

The term “**organohydroxysilane**” refers to a group alkyl–SiOH wherein alkyl is as herein defined.

The term “**patient**” refers to a warm-blooded animal, more preferably to a human, who/which is awaiting the receipt of, or is receiving medical care or is/will be the object
15 of a medical procedure.

The term “**peptide**” refers to a linear polymer of amino acids of less than 50 amino acids linked together by peptide bonds. The term “**pseudopeptide**” refers to a peptide having a modified peptide backbone, namely with at least one peptide bond –[CO–NH] – replaced by a bioisosteric surrogate which is nonhydrolyzable or hydrolyzable only under severe
20 conditions. In the vast majority of published pseudopeptides, only one or a very few peptide bonds had been replaced and most monomeric units are amino acids.

The term “**pharmaceutically acceptable**” means that the ingredients of a pharmaceutical composition are compatible with each other and not deleterious to the patient thereof.

The term “**pharmacologically active substances**” refers to a compound for therapeutic
25 use, and relates to health. Especially, a pharmaceutically active substance may be indicated for treating or preventing a disease. According to the invention, the term “treating a disease” refers to reducing or alleviating at least one adverse effect or symptom of a disease, disorder or condition associated with a deficiency in an organ, tissue or cell

function. The expression “Preventing a disease” or “Inhibiting the development of a disease” refers to preventing or avoiding the occurrence of symptom.

The term “**photothermal therapy**” refers to the use of electromagnetic radiation (most often in infrared wavelengths) for the treatment of various medical conditions, including
5 cancer.

The term “**polyalkoxyalkylene**” refers to a group $-(\text{alkyl}-\text{O})_n-\text{alkyl}-$, wherein alkyl is as herein defined and n represent an integer, preferably ranging from 2 to 100.

The term “**polyamine**” refers to a group comprising at least two amine moieties. Examples of polyamines are diethylenetriamine and spermidine.

10 The term “**polycarboxylic acid**” refers to a chemical moiety comprising at least two carboxylic acid functions. Examples of polycarboxylic acid are citric acid, tartaric acid, glutaric acid, malic acid, tartronic acid, cyclohexanetricarboxylic acid, cyclohexanehexacarboxylic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, 4-bromomandelic acid, cis,cis,cis,cis-1,2,3,4-
15 cyclopentanetetracarboxylic acid, dibenzoyl-D-tartaric acid, chelidonic acid, tetrahydrofuran-1,3,4,5-tetracarboxylic acid, isocitric acid, mucic acid, oxalic acid, glucuronic acid. Preferably the polycarboxylic acid is citric acid.

The term “**polyethylene glycol (PEG)**” refers to a polyether compound with many applications from industrial manufacturing to medicine. Among polyethylene glycols
20 suitable as hydrophilic groups having an effect on biodistribution or on macrophage capture there is for example compounds of formula $\text{R}^1-\text{NH}-\text{R}^2$ wherein R^1 and R^2 are the same or different and represent H, alkyl or a polyethylene glycol chain of formula $-\text{CH}_2-(\text{CH}_2-\text{O}-\text{CH}_2)_k-\text{CH}_2\text{OR}^3$ wherein k is ranging from 2 to 100, and R^3 is selected from H, alkyl and $-(\text{CO})-\text{alkyl}$. Examples of aminopolyethyleneglycols are O-(2-aminoethyl)-O'-
25 methylpolyethyleneglycol 1100, O-(2-aminoethyl)-O'-methylpoly-ethyleneglycol 2000, O-(2-aminoethyl)-O'-methylpolyethyleneglycol 750, PEG 340, PEG 750, PEG 2000, diethylene glycol, tetraethylene glycol, hexaethylene glycol.

The term “**polyphosphoric acid**” refers to a chemical moiety comprising at least two phosphoric acid functions. Examples of polyphosphoric acid are pyrophosphoric acid
30 tripolyphosphoric acid, tetrapolyphosphoric acid, trimetaphosphoric acid,

hexametaphosphoric acid. Preferably the polyphosphoric acid is hexametaphosphoric acid.

The term “**polysaccharide**” refers to a polymeric carbohydrate molecule composed of long chains of monosaccharide units bound together by glycosidic linkages; which may
5 be linear or branched. Examples include starch, glycogen, cellulose and chitin.

The term “**preservative agent**” refers in the present invention to a substance that is added to products such as food, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth.

10 The term “**prevent**”, “**preventing**” and “**prevention**”, as used herein, refer to a method of delaying or precluding the onset of a condition or disease and/or its attendant symptoms, barring a patient from acquiring a condition or disease, or reducing a patient’s risk of acquiring a condition or disease.

The term “**protein**” refers to a functional entity formed of one or more peptides.

15 The term “**radioopacifying agent**” refers to a substance that will not allow X-rays or similar radiation to pass.

The term “**radiosensitizer**” refers to an agent that makes tumor cells or infected cells more sensitive to radiation therapy. It is sometimes also known as a radiation sensitizer or radio-enhancer.

20 The term “**radiotherapy**” refers to therapy using ionizing radiation usually delivered by a linear accelerator, generally as part of cancer treatment to control.

The term “**reducing agent**” refers to a compound that loses (or "donates") an electron to another chemical species in a redox chemical reaction.

The term “**saccharide**” refers to poly- and mono-saccharides as herein defined.

25 The term “**silanization**” refers to the covering of a surface with silica, optionally functionalized silica, using alkoxysilane molecules or using organofunctional alkoxysilane molecules such as alkyl alkoxysilane, chloro alkoxysilane, amino alkoxysilane, epoxy alkoxysilane, fluoro alkoxysilane, isocyanate alkoxysilane, mercapto

alkoxysilane, metacrylate alkoxysilane, oximino alkoxysilane, ammonium alkoxysilane, thiocyanato alkoxysilane, ureido alkoxysilane or vinyl alkoxysilane. After silanization with alkoxysilane molecules, the surface is covered by silica. In case of silanization with organofunctional alkoxysilane molecules, the surface is covered by organohydroxysilane groups, which may optionally be oligomerized.

The terms “**stability**” or “**stable**” in relation with Bi NPs, if not otherwise specified, may refer to two types of stabilities: (1) stability of the NPs with regards to agglomeration and/or aggregation and/or precipitation, i.e. colloidal stability in solution, and (2) stability of the NPs with regards to degradation by dissolution.

Degradation by dissolution may be assessed by UV-visible measurements or by TEM analysis. TEM enables to determine the size of the metallic core of the Bi NP, without taking into account the coating layer. On the contrary, DLS enable to measure the hydrodynamic diameter of the whole particle; including the metallic core, adsorbed molecules thereon and solvation. Therefore, colloidal stability may be assessed by DLS measurements: if agglomeration and/or aggregation occurs, then the size of the particles determined by DLS increases. In case of precipitation, visual observation of the suspension may be sufficient.

The term “**stabilizing group**” refers to a chemical group able to be adsorbed or complexed onto the Bi NP surface in order to stabilize the nanoparticle.

The term “**sterilization**” refers to any process that eliminates, removes, kills, or deactivates all forms of life and other biological agents (such as fungi, bacteria, viruses, spore forms, prions, unicellular eukaryotic organisms such as Plasmodium, etc.) present in a sample. Sterilization can be achieved through various means, including: heat, chemicals, irradiation, high pressure, and filtration. In a specific embodiment of the invention, sterilization is performed under heating.

The term “**therapeutically effective amount**” (or more simply an “**effective amount**”) as used herein means the amount of active agent or active ingredient that is sufficient to achieve the desired therapeutic or prophylactic effect in the patient to which/whom it is administered.

The term “**thioalcohol**” refers to chemical groups comprising at least one thiol group and at least one hydroxyl group. Examples of thioalcohol are mercapto alkyl alcohols such as mercaptoethanol, mercaptopropanol mercaptobutanol or dimercaptopropanol.

The term “**thioamine**” refers to chemical groups comprising at least one thiol group and
5 at least one amine group. An example of thioamine group is cysteine.

The term “**thiocarboxylic acid**” refers to chemical groups comprising at least one thiol group and at least one carboxylic acid group. Examples of thiocarboxylic acid groups are dimercaptosuccinic acid, glutathione, mercaptosuccinic acid, lipoic acid , 2,3-butanedithiol acid, 2,3-dimercaptopropanoic acid, 2,4-dimercaptobutanoic acid, 3,5-
10 dimercaptopentanoic acid, 4,6-dimercaptohexanoic acid, 5,7-dimercaptoheptanoic acid, 6,8-dimercaptotanoic acid, 8,10-dimercaptodecanoic acid, 1,2-dithiolane-3-undecanoic acid.

The term “**treat**”, “**treating**” and “**treatment**”, as used herein, are meant to include alleviating, attenuating or abrogating a condition or disease and/or its attendant
15 symptoms.

The term “**ultrafiltration**” refers to a type of filtration, more precisely of membrane filtration, in which forces like pressure lead to a separation through a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained in the so-called retentate, while solvent such as water and low molecular weight solutes pass
20 through the membrane in the permeate (filtrate). Ultrafiltration membranes are defined by the molecular weight cut-off of the membrane used.

The term “**water-soluble**” refers to a substance which is soluble in water.

The term “**zwitterionic**” refers to a group with two or more functional groups, of which at least one has a positive electrical charge and at least another has a negative electrical
25 charge (also called inner salt) and having a stabilization effect. In particular, zwitterionic group refers to sulfobetain groups and amino carboxylic groups as described in Susumu JACS 2011, 9480-9496 and Muro JACS2010, 4556-4557.

As commonly admitted in the art, in the present application, the expressions “greater than X” and “smaller than X” mean that the value X is not included in referred range.

DETAILED DESCRIPTION

Process for manufacturing bismuth nanoparticles

The present invention relates to a process for manufacturing bismuth (0) nanoparticles in aqueous solution. Especially, the process of the invention is performed in aqueous solution, using a water-soluble bismuth precursor, a water-soluble reducing agent and optionally a coating agent.

Advantageously, the water-soluble bismuth precursor, the water-soluble reducing agent and the optional coating agent are totally dissolved in the aqueous solution used for the manufacturing of the bismuth (0) nanoparticles. This is particularly interesting in order to be able to carry out the process in continuous flow.

In one embodiment, the process of the invention is a process for manufacturing biocompatible bismuth (0) nanoparticles comprising a core comprising bismuth (0), surrounded by at least one coating layer; characterized in that it comprises the steps of:

a) preparing an aqueous solution with a pH greater than 7, preferably greater than 12, said aqueous solution comprising:

- a water-soluble bismuth precursor of formula Bi(III)-L ;
- a water-soluble reducing agent;
- optionally one or more coating agent of formula X-L' ; and
- water;

wherein:

X is a cation, preferably a pharmaceutically acceptable cation, more preferably X is a cation selected from hydrogen cation, sodium and ammonium; and

L and L' are each independently a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, and biotargeting derivatives thereof of formula $\text{L}^1\text{-A-L}^2$, wherein:

L^1 is selected from polycarboxylic acid, thiocarboxylic acid and thioalcohol,

L² is a biotargeting group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, such as for example aminoalcohol, polyethylene glycol or zwitterionic groups; and
- a bioactive group, such as for example antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances; and

A is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;

b) heating the aqueous solution obtained in step a), and

c) recovering the bismuth (0) nanoparticles from the aqueous solution obtained in step b).

In one embodiment, the process of the invention enables to manufacture biocompatible bismuth (0) nanoparticles comprising a core comprising bismuth (0), surrounded by at least one coating layer adsorbed thereon.

In one embodiment, the water-soluble bismuth precursor is totally dissolved in the
5 solution prepared in step a).

In one embodiment, the water-soluble bismuth precursor of formula Bi(III)-L, wherein L is a stabilizing group selected from polycarboxylic acid and thiocarboxylic acid. In one embodiment, the water-soluble bismuth precursor is selected from bismuth (III) citrate, bismuth (III) lipoate, bismuth (III) dimercaptosuccinate, bismuth (III)
10 dimercaptopropanol and bismuth (III) mercaptoethanol; preferably the water-soluble bismuth precursor is selected from bismuth (III) citrate, bismuth (III) lipoate and bismuth (III) dimercaptosuccinate; more preferably the water-soluble bismuth precursor is bismuth (III) citrate.

In one embodiment, the water-soluble reducing agent is selected from saccharides,
15 preferably monosaccharides; preferably the water-soluble reducing agent is glucose.

In one embodiment, step a) is conducted in absence of coating agent. This is preferably the case when the process is conducted in batch. In such case, the Bi NPs are stabilized by the counter ion L of the bismuth precursor.

In another embodiment, step a) is conducted in presence of one or more coating agent.
20 This is preferably the case when the process is conducted in continuous flow. In such case, preferably one, two or three coating agents may be used. When only one coating agent is used, L' may be identical or different from counter ion L of the bismuth precursor.

In a specific embodiment, the cation X is a pharmaceutically acceptable cation. Preferably X is selected from hydrogen cation, sodium, ammonium, aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium,
25 meglumine, olamine, potassium, tromethamine, 2-(diethylamino)ethanol, ethanolamine, morpholine, 4-(2-hydroxyethyl)morpholine and zinc cations. More preferably, X is selected from hydrogen cation, sodium and ammonium. In a specific embodiment, the

cation X is hydrogen cation. In another specific embodiment, the cation X is ammonium. In another specific embodiment, the cation X is sodium.

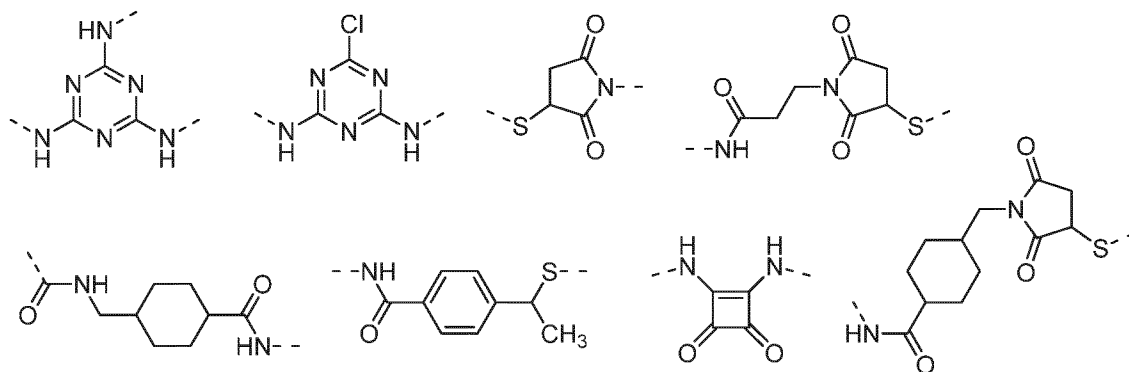
In one embodiment, L, L' and L¹ are each independently selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol. Preferably, the polycarboxylic acid is citric, tartaric, 5 tartronic or oxalic acid, preferably citric acid; the thiocarboxylic acid is dimercaptosuccinic acid, glutathione, lipoic acid or 2,3-dimercapto propanoic acid; and the thioalcohol is dimercaptopropanol or 2-mercaptoethanol. In one embodiment, L, L' and L¹ are respectively selected from citric acid, lipoic acid, dimercaptosuccinic acid, glutathione, dimercaptopropanol, 2-mercaptoethanol and biotargeting derivatives thereof.

10 In one embodiment, the biotargeting group L² is a hydrophilic group having an effect on biodistribution or on macrophage capture. In such case, L² is preferably selected from aminoalcohol groups, polyethylene glycol groups or zwitterionic groups.

In another embodiment, the biotargeting group L² is a bioactive group. For example, the bioactive group may be selected from antibody, such as polyclonal or monoclonal 15 antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances.

In one embodiment, when L or L' is a biotargeting group of formula L¹-A-L², L¹ comprises at least one linking part to the bismuth metallic (0) core of the nanoparticle and 20 at least one covalent coupling function to the biotargeting group L² via the linking group A.

The linking groups A or I (present when A is -P¹-I-P²) are advantageously of the type of: -CONH-, -COO-, -NHCO-, -OCO-, -NH-CS-NH-, -C-S-, -N-NHCO-, -CO-NH-N-, -CH₂-NH-, -N-CH₂-, -N-CS-N-, -CO-CH₂-S-, -N-CO-CH₂-S-, -NCO-CH₂-CH₂-S-, 25 -CH=NH-NH-, -NH-NH=CH-, -CH=NO-, -ON=CH- or corresponding to the following formulae:



When the divalent groups mentioned above as possible groups A or I are not symmetrical, it is understood that the group L^1 - or L^1 -P¹- may be grafted on the right or on the left of said covalent group. For example, when A is indicated to represents a group -COO-, the corresponding stabilizing group may be L^1 -COO- L^2 or L^2 -COO- L^1 .

In one embodiment, when L' is a biotargeting group of formula L^1 -A- L^2 in the coating agent, then the coating agent is of formula X- L^1 -A- L^2 .

In one embodiment, the pH of the aqueous solution is greater than 7 and is adapted to the water-soluble reducing agent used in the process. In a particularly preferred embodiment, the pH of the aqueous solution is greater than 12. In a specific embodiment, the water-soluble reducing agent is glucose and the pH of the aqueous solution of step a) is greater than 12, preferably the pH is ranging from 12 to 14, more preferably from 12.5 to 13.7.

According to one embodiment, the molar ratio of the water-soluble reducing agent to the water-soluble bismuth precursor is ranging from 0.4 to 5, preferably from 1 to 3.5. In a specific embodiment, the bismuth nanoparticles are prepared in batch and the molar ratio of the water-soluble reducing agent to the water-soluble bismuth precursor is ranging from 1 to 2. In an alternative specific embodiment, the bismuth nanoparticles are prepared in continuous flow and the molar ratio of the water-soluble reducing agent to the water-soluble bismuth precursor is ranging from 1 to 3.5. In a specific embodiment, the water-soluble reducing agent is glucose and the water-soluble bismuth precursor is bismuth citrate, and in this case, the molar ratio of the water-soluble reducing agent to the water-soluble bismuth precursor is preferably 1.5 in batch and 3.5 in continuous flow.

According to one embodiment, the molar ratio of the coating agent to the water-soluble bismuth precursor is ranging from 0 to 25, preferably from 0 to 20. In a specific embodiment, no coating agent is used in the process of the invention; preferably in the case of a preparation in batch. In a specific embodiment, the bismuth nanoparticles are prepared in batch and the molar ratio of the coating agent to the water-soluble bismuth precursor is ranging from 0 to 2. In an alternative specific embodiment, the bismuth nanoparticles are prepared in continuous flow and the molar ratio of the coating agent to the water-soluble bismuth precursor is ranging from 5 to 20.

According to one embodiment, the concentration in water-soluble bismuth precursor in the aqueous solution is ranging from 0.5 mM to 100 mM, preferably from 1 mM to 20 mM, more preferably from 2 mM to 15 mM in batch. In a specific embodiment, the bismuth nanoparticles are prepared in batch and the concentration in water-soluble bismuth precursor in the aqueous solution is ranging from 0.5 mM to 100 mM, preferably from 5 mM to 20 mM, more preferably from 7 mM to 15 mM. In an alternative specific embodiment, the bismuth nanoparticles are prepared in continuous flow and the concentration in water-soluble bismuth precursor in the aqueous solution is ranging from 0.5 mM to 100 mM, preferably from 1 mM to 10 mM, more preferably from 2 mM to 5 mM. In a specific embodiment, the water-soluble reducing agent is glucose and the water-soluble bismuth precursor is bismuth citrate, and in this case, the preferred concentration in bismuth citrate in the aqueous solution is equal to 10 mM in batch and 3 mM in continuous flow.

According to one embodiment, the aqueous solution comprises one coating agent. In an alternative embodiment, the aqueous solution comprises at least two different coating agents. In such case, the aqueous solution may comprise a non-functionalized coating agent and a functionalized biotargeting coating agent. In another embodiment, the aqueous solution may comprise a coating agent functionalized by a hydrophilic group or a zwitterionic group and a coating agent functionalized by a bioactive group.

According to one embodiment, the coating agent is a biotargeting coating agent as those disclosed in FR2 921 837.

In one embodiment, the heating of step b) is performed by classical thermal heating, by irradiation under microwaves or by ultrasonication. Preferably the heating of step b) is performed by irradiation under microwaves for batch production and by classical thermal heating for continuous flow production.

- 5 According to one embodiment, the heating of step b) is performed by classical thermal heating at a temperature ranging from 50°C to 150°C, preferably from 80°C to 120°C. According to one embodiment, the heating of step b) is performed by classical thermal heating during a period of time ranging from 1 hour to 5 hours, preferably from 2 hours to 4 hours, more preferably from 2h30 to 3h30 in batch.

- 10 According to a specific embodiment, the heating of step b) is performed by classical thermal heating without stirring the reaction medium.

According to one embodiment, the heating of step b) is performed by classical thermal heating during a residence time ranging from 1 min to 120 min, preferably from 1 min to 30 min, more preferably from 5 min to 15 min, for continuous flow production.

- 15 According to one embodiment, the heating of step b) is performed by irradiation under microwaves. Preferably, irradiation under microwaves is performed at a temperature higher than 70°C, preferably ranging from 70°C to 160°C, more preferably from 90°C to 150°C, more preferably from 100°C to 140°C.

- 20 According to one embodiment, the heating of step b) is performed by irradiation under microwave during a period of time ranging from 0.5 min to 15 min, preferably from 1 min to 10 min, more preferably from 1 min to 8 min.

According to one embodiment, the heating of step b) is performed by irradiation under microwave with an irradiation power ranging from 25 W to 150 W, preferably 50 W to 120 W, more preferably lower than 100 W.

- 25 In one embodiment, the recovering of step c) is made by filtration, preferably by ultrafiltration, more preferably by ultrafiltration with filters ranging from 1 kDa to 100 kDa. In a specific embodiment, the bismuth nanoparticles are prepared in batch and

filtration is performed by ultrafiltration with 3 kDa filters. In an alternative embodiment, the bismuth nanoparticles are prepared in continuous flow and filtration is performed by ultrafiltration with 30 kDa filters. Ultrafiltration advantageously enables to retrieve higher percentages of monodisperse populations of nanoparticles with small sizes.
5 Moreover, ultrafiltration is more easily scalable than centrifugation.

In one embodiment, the process of the invention further comprises a step of sterilization, preferably a step of sterilization under heating. The nanoparticles obtained by the process of the invention present the advantage to be stable to such a sterilization treatment. In a specific embodiment, the sterilization under heating is performed at a temperature of
10 more than 100°C, for example at a temperature ranging from 100°C to 150°C, preferably at about 121°C. In a specific embodiment, the sterilization under heating is performed for a period of time ranging from 5 minutes to 2 hours, preferably from 5 minutes to 1 hour, preferably from 5 minutes to 30 minutes, more preferably for about 20 minutes. Preferably sterilization is performed by moist heat, preferably by moist heat at 121°C. In
15 a specific embodiment, sterilization is performed by moist heat at 121°C for about 20 minutes.

In one embodiment, the yield of synthesis of the Bi NPs by the process of the invention is of more than 60%, preferably more than 70%, more preferably more than 80%. Advantageously, when the process of the invention is conducted in batch, the yield is of
20 more than 80%, preferably more than 90%, more preferably more than 95%.

In a preferred embodiment, the process of the invention provides bismuth (0) nanoparticles coated with citrate as stabilizing group. The citrate stabilizing group may either come from the bismuth precursor or from the coating agent.

In one embodiment, the process of the invention comprises an additional step of coating-exchange. This additional step enables to provide Bi NPs with a wide variety of coating,
25 especially with hydrophilic, hydrophobic, amphiphilic or fluorophilic coatings. In an embodiment, the coating-exchange is performed by contacting the bismuth (0) nanoparticles obtained in step c) with a coating agent if none was used in the process or

with a further coating agent if one or more coating agent was already used in the process. Preferably the coating agent for the coating exchange is of formula $X''-L''$, wherein:

X'' is a cation, preferably a pharmaceutically acceptable cation, more preferably a cation selected from hydrogen cation sodium and ammonium; and

5 L'' is a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, polysaccharide, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$; preferably L'' is a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid, cyclodextrine
10 and derivatives thereof of formula $L^{1''}-A''-L^{2''}$; wherein:

$L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, polysaccharide, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine;
15 preferably $L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine; and

$L^{2''}$ is a group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage
20 capture, such as for example aminoalcohol, polyethylene glycol or zwitterionic groups;
- a bioactive group, such as for example antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody;
25 peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, such as for example saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and

- a fluorophilic group capable of solubilizing Bi NPs in an fluorous phase, such as for example saturated or unsaturated C₃F₇ to C₁₈F₃₇ perfluorinated chains or partially fluorinated alkyl chains; and
- A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-.

Specific embodiments disclosed above for L, L', L¹, L² and A also apply here by analogy to L'', L^{1''}, L^{2''} and A''.

In one embodiment, L'' and L^{1''} are respectively selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, polysaccharide, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine; preferably L'' and L^{1''} are respectively selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine. Preferably, the polycarboxylic acid is citric, tartaric, tartronic or oxalic acid, preferably citric acid; the thiocarboxylic acid is lipoic acid, glutathione, dimercaptosuccinic acid, 2,3-dimercapto propanoic acid, cysteine or acetyl cysteine; the thioalcohol is dimercaptopropanol or 2-mercaptoethanol; the thioamine is cysteine; the polyamine is diethylenetriamine or spermidine; the polysaccharide is pectin;

the aminosaccharide is glucosamine; and the polyphosphoric acid is hexametaphosphoric acid. In one embodiment, L'' and L^{1''} are respectively selected from citric acid, lipoic acid, glutathione, dimercaptosuccinic acid, cysteine, acetyl cysteine; dimercaptopropanol, 2-mercaptoethanol, diethylenetriamine, spermidine, pectin, glucosamine, diphosphonopentanoic acid, hexametaphosphic acid, cyclodextrine and biotargeting derivatives thereof. In one embodiment, L'' and L^{1''} are respectively selected from citric acid, lipoic acid, glutathione, dimercaptosuccinic acid, cysteine, acetyl cysteine; dimercaptopropanol, 2-mercaptoethanol, diethylenetriamine, spermidine, glucosamine, diphosphonopentanoic acid, hexametaphosphic acid, cyclodextrine and biotargeting derivatives thereof.

In one embodiment, L^{2''} is a hydrophobic group capable of solubilizing Bi NPs in an oily phase. For example, the hydrophobic group may be a saturated or unsaturated C₆H₁₃ to C₁₈H₃₇ fatty alkyl chain such as dodecyl, tetradecyl, hexyldecyl or octadecyl. In a specific embodiment, the stabilizing group L'' is a lipid acid coupled by amide coupling with dodecylamine, tetradecylamine, hexyldecylamine or octadecylamine. In a specific embodiment, the stabilizing group L'' is a lipid acid coupled by amide coupling with stearyl amine or with lysine and further with stearyl amine, as disclosed in Segota et al. (The Journal of Physical Chemistry, Vol. 119, 2015, pp. 5208-5219).

In one embodiment, L^{2''} is a fluorophilic group capable of solubilizing Bi NPs in a fluororous phase. For example, the fluorophilic group may be a saturated or unsaturated C₃F₇ to C₁₈F₃₇ perfluorinated chain, such as 1,1,2,2,3,3,3-heptafluoropropyl, 1,1,2,2,3,3,4,4,4-nonafluorobutyl or perfluoropentyl; or a partially fluorinated alkyl chain, such as 2,2,2-trifluoroethyl, 2,2,3,3,3-pentafluoropropyl, 2,2,3,3,4,4,4-heptafluorobutyl, 2,2,3,3,4,4,5,5,6,6,6-underdecafluorohexyl, 4,4,5,5,6,6,6-heptafluorohexyl, 3,3,4,4,5,5,6,6,7,7,7-underfluoroheptyl or 5,5,6,6,6-pentafluorohexyl. In a specific embodiment, the stabilizing group L'' is a lipid acid coupled by amide coupling with 1,1,2,2,3,3,3-heptafluoropropan-1-amine, 1,1,2,2,3,3,4,4,4-nonafluorobutan-1-amine, 1-(perfluoropentyl)amine, 2,2,2-trifluoroethylamine, 2,2,3,3,3-pentafluoropropylamine, 2,2,3,3,4,4,4-heptafluorobutyl-1-amine,

2,2,3,3,4,4,5,5,6,6,6-underdecafluorohexylamine, 4,4,5,5,6,6,6-heptafluorohexylamine, 3,3,4,4,5,5,6,6,7,7,7-underfluoroheptylamine or 5,5,6,6,6-pentafluorohexylamine.

According to one embodiment, the coating-exchange step is performed in aqueous solution. According to one embodiment, the coating-exchange step is performed under heating, for example by classical thermal heating, by irradiation under microwaves or by ultrasonication. According to one embodiment, after the coating-exchange, the Bi NPs are recovered as described previously. According to an alternative embodiment, the coating-exchange step is performed at room temperature.

In a preferred embodiment, the process of the invention first provides bismuth (0) nanoparticles coated with citrate as stabilizing group, and then a coating exchange is performed using a functionalized lipoic acid derivative as coating agent, preferably lipoic acid functionalized with a hydrophilic, hydrophobic, bioactive or fluorophilic group.

In another preferred embodiment, the process of the invention first provides bismuth (0) nanoparticles coated with citrate as stabilizing group, and then a coating exchange is performed using a functionalized citric acid derivative as coating agent, preferably citric acid functionalized with a hydrophilic, hydrophobic, bioactive or fluorophilic group as described in FR2 921 837.

In one embodiment, the process of the invention comprises an additional step of silanization. In an embodiment, the silanization is performed by contacting the bismuth (0) nanoparticles obtained in step c) with tetraethyl orthosilicate (TEOS), (triethoxysilyl)propylsuccinic anhydride (TEPSA), (3-aminopropyl)triethoxysilan (APTES), or with other reactants suitable for silanization known in the art.

Alternatively, a coating-exchange step as described above may be performed before performing the silanization step.

Optionally, the silanization step may be followed by a functionalization step. Especially, silica on the nanoparticles may be functionalized by an organohydroxysilane group functionalized by:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, such as for example aminoalcohol, polyethylene glycol or zwitterionic groups; and
- a bioactive group, such as for example antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, such as for example organohydroxysilane groups such as alkyl hydroxysilane, metacrylate hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octohydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane, octodecylhydroxysilane or methacryloxypropylhydroxysilane; and
- a fluorophilic group capable of solubilizing Bi NPs in an fluorous phase, such as for example perfluorinated organohydroxysilane groups or partially fluorinated alkylhydroxysilane groups such as fluoromethylhydroxysilane, 2-fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxysilane, 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-pentafluorobutylhydroxysilane.

In one embodiment, the functionalization step leading to the functionalization by a hydrophobic group may be performed using organoalkoxysilane compounds such as for example alkoxysilane groups such as alkyl alkoxysilane, metacrylate alkoxysilane or ureido alkoxysilane; vinyl alkoxysilane groups such as ethyltrimethoxysilane, propyltrimethoxysilane, butyltrimethoxysilane, pentyltrimethoxysilane,

hexyltrimethoxysilane, heptyltrimethoxysilane, octotrimethoxysilane,
 nonyltrimethoxysilane, decyltrimethoxysilane, dodecyltrimethoxysilane,
 tetradecyltrimethoxysilane, hexyldecyltrimethoxysilane, octodecyltrimethoxysilane or
 methacryloxypropyltrimethoxysilane. The resulting organohydroxysilane groups present
 5 on the nanoparticle may be oligomerized.

In one embodiment, the functionalization step leading to the functionalization by a
 fluorophilic group may be performed using a perfluorinated organoalkoxysilane
 compound or a partially fluorinated alkylalkoxysilane compound such as for example
 fluoromethyltrimethoxysilane 2-fluoroethyltrimethoxysilane, 2,2-
 10 difluoroethyltrimethoxysilane, 2,2,2-trifluoroethylmethoxysilane,
 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluorooctyltriethoxysilane, 3,3,4,4,5,5,6,6,6-
 nonafluorohexyltriethoxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptyltriethoxysilane,
 2,2,3,3,3-pentafluoropropyltriethoxysilane, 3,3,4,4,5,5,5-
 heptafluoropentyltrimethoxysilane, 3,3,4,4,4-pentafluorobutyltriethoxysilane. The
 15 resulting perfluorinated organohydroxysilane groups present on the nanoparticle may be
 oligomerized.

In a preferred embodiment, the process of the invention first provides bismuth (0)
 nanoparticles coated with citrate as stabilizing group, and then a silanization step is
 performed followed by a functionalization step of using siloxane groups functionalized
 20 with hydrophilic, hydrophobic or fluorophilic groups.

The process of the invention presents the advantage to be conducted in green conditions,
 especially as being conducted in water. Advantageously, the process of the invention may
 be conducted under ambient atmosphere while previously known methods of
 manufacturing of bismuth nanoparticles required to be conducted under inert gaz.

25 In one embodiment, the process of the invention is carried out in continuous flow. A
 continuous flow process presents the advantage of being easily scalable and reproducible.

In one embodiment, the bismuth (0) nanoparticles obtained by the process of the
 invention are monodisperse.

Bismuth nanoparticles

The present invention also relates to biocompatible bismuth (0) nanoparticles, especially biocompatible bismuth (0) nanoparticles obtainable by the process of the invention.

In one embodiment, the bismuth (0) nanoparticles of the invention comprise a core comprising bismuth (0), surrounded by at least one coating layer wherein the coating layer is adsorbed on the core.

In one embodiment, the bismuth (0) nanoparticle comprises:

- a core comprising bismuth (0),
- surrounded by at least one coating layer comprising:
 - (1) at least one a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, polysaccharide, aminosaccharide, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$; preferably the stabilizing group is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$; wherein:
 - $L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, polysaccharide, aminosaccharide, polyphosphoric acid and cyclodextrine; preferably $L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid and cyclodextrine; and
 - $L^{2''}$ is a group selected from:
 - a hydrophilic group having an effect on biodistribution or on macrophage capture, such as for example aminoalcohol, polyethylene glycol or zwitterionic groups;
 - a bioactive group, such as for example antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;

- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, such as for example saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and
- a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, such as for example saturated or unsaturated C_3F_7 to $C_{18}F_{37}$ perfluorinated chains or partially fluorinated alkyl chains; and

A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;

and/or

(2) at least one organohydroxysilane group functionalized by a group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, such as for example aminoalcohol, polyethylene glycol or zwitterionic groups;
- a bioactive group, such as for example antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or

- minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, such as for example hydroxysilane groups such as alkyl hydroxysilane, metacrylate hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octohydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane, octadecylhydroxysilane or methacryloxypropylhydroxysilane; and
 - a fluorophilic group capable of solubilizing Bi NPs in an fluorous phase, such as for example perfluorinated organohydroxysilane groups or partially fluorinated alkylhydroxysilane groups such as fluoromethylhydroxysilane 2-fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxysilane, 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-pentafluorobutylhydroxysilane.

According to one embodiment, the Bi NP comprises one coating layer. According to another embodiment, the Bi NP comprises at least two coating layers. In one embodiment, the coating layer is a continuous layer. In an alternative embodiment, the coating layer is a discontinuous layer. As mentioned above, the coating layer is adsorbed on the core of the nanoparticle.

According to one embodiment, the Bi NP comprises one coating agent.

In an alternative embodiment, the Bi NP comprises at least two different coating agents. In such case, at least one of the coating agent may be non-functionalized and at least another coating agent may be a functionalized biotargeting agent.

5 In another embodiment, the Bi NP may comprise a coating agent functionalized by a hydrophilic group and another coating agent functionalized by a bioactive group. In such case, the coating agent functionalized by the hydrophilic group may bear a group to modulate macrophage capture and the coating agent functionalized by a bioactive group may have a specific affinity for a given target, this combination leading to an improved biodistribution of the nanoparticle.

10 In another embodiment, the Bi NP may comprise two different coating agents functionalized by different bioactive group. In such case, the two different bioactive groups may target the same pathology (for example two peptides targeting respectively a first receptor overexpressed in tumor cells and a second type of receptor overexpressed in the same tumor cells) or different pathologies.

15 In one embodiment, the stabilizing group is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, polysaccharide, aminosaccharide, polyphosphoric acid, cyclodextrine; preferably the stabilizing group is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid, cyclodextrine. Preferably, the polycarboxylic acid is citric, tartaric, tartronic or oxalic acid, preferably citric acid; the thiocarboxylic acid is lipoic acid, glutathione, dimercaptosuccinic acid, 2,3-dimercapto propanoic acid, cysteine or acetyl
20 cysteine; the thioamine is cysteine; the polyamine is diethylenetriamine or spermidine; the polysaccharide is pectin; the aminosaccharide is glucosamine; and the polyphosphoric acid is hexametaphosphoric acid. In one embodiment, the stabilizing group is selected
25 from citric acid, lipoic acid, glutathione, dimercaptosuccinic acid, cysteine, acetyl cysteine; diethylenetriamine, spermidine, pectin, glucosamine, hexametaphosphonic acid, cyclodextrine and derivatives thereof; preferably the stabilizing group is selected from citric acid, lipoic acid, glutathione, dimercaptosuccinic acid, cysteine, acetyl cysteine; diethylenetriamine, spermidine, glucosamine, hexametaphosphonic acid,
30 cyclodextrine and derivatives thereof.

According to one embodiment, the nanoparticle comprises from 1 to 100 stabilizing groups per nm²; preferably from 1 to 70 stabilizing groups per nm²; more preferably from 1 to 50 stabilizing groups per nm²; more preferably from 1 to 40 stabilizing groups per nm².

- 5 According to one embodiment, the size of the bismuth (0) nanoparticles of the invention is smaller than 300 nm measured by Transmission Electron Microscopy (TEM), preferably smaller than 200 nm, more preferably smaller than 150 nm, more preferably smaller than 100 nm, more preferably smaller than 50 nm, smaller than 40 nm, smaller than 30 nm, smaller than 20 nm, smaller than 15 nm. The TEM size reflects the size of
10 the metallic bismuth core of the nanoparticles.

- In one embodiment, the bismuth (0) nanoparticles are obtained by the process of the invention performed in continuous flow and in this case the size of the bismuth (0) nanoparticles of the invention is smaller than 80 nm, preferably smaller than 50 nm, more preferably smaller than 20 nm, and even more preferably smaller than 10 nm, measured
15 by TEM.

- According to one embodiment, the size of the bismuth (0) nanoparticles of the invention is smaller than 400 nm measured by dynamic light scattering (DLS), preferably smaller than 300 nm, preferably smaller than 250 nm, more preferably smaller than 200 nm, more preferably smaller than 150 nm. The DLS size reflects the hydrodynamic diameter of the
20 nanoparticle.

- In one embodiment, the bismuth (0) nanoparticles are obtained by the process of the invention performed in continuous flow and in this case the size of the bismuth (0) nanoparticles of the invention is smaller than 200 nm, preferably smaller than 150 nm, more preferably smaller than 100 nm, and even more preferably smaller than 75 nm,
25 measured by DLS.

In one embodiment, the ratio of the nanoparticle hydrodynamic diameter measured by DLS in number to the nanoparticle size measured by TEM is ranging from 1 to 90, preferably from 1 to 70, preferably from 1 to 50, preferably from 1 to 45, preferably from

1 to 30, preferably from 1 to 25 preferably from 1 to 20. Such a low ratio reflects the fact that the nanoparticle comprises a high bismuth payload, which is advantageous to maximize X-ray opacity and thus limit the amount of nanoparticles needed for imaging.

In one embodiment, the bismuth (0) nanoparticles do not contain boron. Especially, the bismuth (0) nanoparticles do not comprise boron derivatives, such as boranes, borates, boric acid, boronic acid, borinic acid, boronates, borohydrides, salts and derivatives thereof. By “borate” is made reference to salts or esters of boric acid ($B(OH)_3$), boronic acid ($RB(OH)_2$) or borinic acid ($R_2B(OH)$). By “borane” is made reference to BR_3 wherein R is alkyl or H. By “borohydride” is made reference to the anion BH_4^- and its salts. Borohydride is also the term used for compounds containing $BH_{4-n}X_n^-$, wherein n is an integer and X an anion, such as for example cyanoborohydride ($B(CN)H_3^-$) and triethylborohydride ($B(C_2H_5)_3H^-$). Especially, this means that the nanoparticles of the invention are not synthesized in presence of boranes or borohydride reducing agents. The absence of derivatives of bore is advantageous since these compounds are known to be toxic and should thus be avoided, especially for in vivo applications.

In one embodiment, the bismuth (0) nanoparticles are monodisperse.

In one embodiment, the bismuth (0) nanoparticles are of spherical shape.

According to one embodiment, the bismuth (0) nanoparticles are stable. Especially, when the nanoparticles are dispersed in a solvent, they remain stable to agglomeration and/or aggregation and/or precipitation, as well as stable to dissolution. Moreover, the nanoparticles of the invention also remain stable in solid for.

According to a specific embodiment, the nanoparticles remain stable in their dispersion solvent at a concentration greater than 1 mM with regards to agglomeration and/or aggregation and/or precipitation for at least 30 minutes; preferably for at least 1 hour; or preferably for at least 2 hours.

The choice of the dispersion solvent of the nanoparticles depends on the functionalities present on the coating layer(s) of said nanoparticles. For example, if the nanoparticle has a coating layer comprising hydrophilic groups, the dispersion solvent would be an

aqueous medium; while if the coating layer comprises hydrophobic groups or fluorophilic groups, the dispersion solvent would be for example an oil or a fluoruous phase, respectively.

According to a specific embodiment, the nanoparticles remain stable in their dispersion solvent at a concentration greater than 1 mM with regards to dissolution for at least 1 day; preferably at least 10 days, more preferably at least 1 month.

According to a specific embodiment, the nanoparticles remain stable in solid form for at least 1 week; preferably at least 1 month, more preferably at least 2 months, even more preferably for at least 3 months.

10 According to one embodiment, when the bismuth (0) nanoparticles of the invention are soluble in water, they are stable in water at pH 7 at a concentration greater than 1 mM for at least 1 month, with regards to stability to agglomeration and/or aggregation and/or precipitation and stability to dissolution.

15 In one embodiment, the bismuth (0) nanoparticles of the invention may be soluble in water when the coating layer comprises at least one a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, polysaccharide, aminosaccharide, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$, wherein $L^{1''}$ and A'' are as defined above and $L^{2''}$ is a hydrophilic group or a bioactive group, as defined above; preferably $L^{2''}$ is a hydrophilic group.

20 According to one embodiment, the bismuth (0) nanoparticles of the invention are soluble in oil phase, i.e. coated by hydrophobic group, and are stable in oil phase or organic solvent at a concentration greater than 1 mM for at least 1 month.

According to one embodiment, the bismuth (0) nanoparticles of the invention are soluble in fluoruous phase, i.e. coated by fluorophilic group, and are stable in fluoruous phase at a concentration greater than 1 mM for at least 1 month.

25 According to one embodiment, the bismuth (0) nanoparticles of the invention are stable in solid form for at least 3 months.

According to one embodiment, the bismuth (0) nanoparticles of the invention are stable with regards to sterilization process, especially sterilization under heating as described above.

According to one embodiment, the bismuth (0) nanoparticles of the invention are silanized. In such case, the DLS size of the silanized nanoparticles is increased from 10 to 50% more preferably from 15% to 30%.

Optionally, the silanized bismuth (0) nanoparticles are functionalized. Especially, silica on the nanoparticles may be functionalized by an organoalkoxysilane group functionalized as described above.

Silanized Bi NPs are especially interesting for incorporation of the nanoparticles in emulsion, especially in oil-in-water emulsions, in polymers and in cements such as for examples in dental cements.

Composition / Formulations

The present invention also relates to a composition comprising bismuth (0) nanoparticles of the invention and a carrier.

The composition may be for example a solution, preferably an aqueous solution; a suspension; an emulsion, including oil-in-water emulsion or water-in-oil emulsion; a cement composite resin; or a hydrogel.

According to one embodiment, in the composition of the invention, the concentration of bismuth (0) nanoparticles is ranging from 0.2 mM to 2000 mM, preferably from 0.2 mM to 500 mM.

In one embodiment, in the composition of the invention, the carrier is a pharmaceutically acceptable carrier. Thus the invention also relates to a pharmaceutical composition comprising bismuth (0) nanoparticles of the invention in association with at least one pharmaceutically acceptable carrier.

Generally, for pharmaceutical use, the Bi NPs of the invention may be formulated as a pharmaceutical preparation comprising Bi NPs of the invention and at least one pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant, and optionally one or more further pharmaceutically active compounds.

- 5 By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular, intradermic or subcutaneous injection or intravenous infusion), for intralesional administration, for submucosal administration, for intra-articular administration, for intra-tumoral administration for intra-cavitary administration, for topical administration
10 (including ocular), for artery embolization, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc. Such suitable administration forms – which may be solid, semi-solid or liquid, depending on the manner of administration – as well as methods and carriers, diluents and excipients for use in the preparation thereof, will be clear to the skilled person; reference is made to the latest edition of Remington's
15 Pharmaceutical Sciences.

- Some preferred, but non-limiting examples of such preparations include tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, ointments, creams, lotions, soft and hard gelatin capsules, suppositories, drops, sterile injectable solutions and sterile packaged powders (which are usually reconstituted
20 prior to use) for administration as a bolus and/or for continuous administration, which may be formulated with carriers, excipients, and diluents that are suitable for such formulations, such as salts (especially NaCl), glucose, lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, polyethylene glycol, cellulose,
25 (sterile) water, methylcellulose, methyl- and propylhydroxybenzoates, talc, magnesium stearate, edible oils, vegetable oils and mineral oils or suitable mixtures thereof. The formulations can optionally contain other substances that are commonly used in pharmaceutical formulations, such as buffers, antioxidants, lubricating agents, wetting agents, emulsifying and suspending agents, dispersing agents, disintegrants, bulking
30 agents, fillers, preserving agents, sweetening agents, flavoring agents, flow regulators,

release agents, etc.. The compositions may also be formulated so as to provide rapid, sustained or delayed release of the active compound(s) contained therein.

The pharmaceutical preparations of the invention are preferably in a unit dosage form, and may be suitably packaged, for example in a box, blister, vial, bottle, sachet, ampoule
5 or in any other suitable single-dose or multi-dose holder or container (which may be properly labeled); optionally with one or more leaflets containing product information and/or instructions for use.

In one embodiment, the Bi NPs of the invention are formulated in sustained release systems, such as for example encapsulating systems as liposomes or solid lipid
10 nanoparticles, nanospheres, nanocapsules.

According to one embodiment, the composition of the invention is an emulsion, preferably an oil-in-water emulsion.

According to one embodiment, the bismuth (0) nanoparticles of the invention coated by hydrophobic group are incorporated in oil-in-water emulsion according to compositions
15 and processes described for example in WO 2014114724 A1.

According to one embodiment, the bismuth (0) nanoparticles of the invention coated by fluorophilic group are incorporated in (fluorous oil)-in-water emulsion according to compositions and processes described for example in WO 2013045504A1.

The invention also provides a delivery device comprising a container and an injection
20 device, the said container containing a composition rising bismuth (0) nanoparticles of the invention.

Uses

The Bi NPs of the invention may be used in several applications. For example, the Bi NPs of the invention may be used in several fields, such as for example biomedical imaging,
25 medicine, dental, cosmetics, devices, inks.

In one embodiment, the Bi NPs of the invention are useful in biomedical imaging, especially for X-ray, photoacoustic, photothermal and fluorescence imaging.

In one embodiment, the Bi NPs of the invention are useful as X-ray contrast agents. In a preferred embodiment, the Bi NPs of the invention are useful as X-ray contrast agents in
5 X-ray imaging or in photoacoustic.

In one embodiment, the Bi NPs of the invention are useful as radioopacifying agents, especially in the field of biomedical devices, for example to render dental cements, implants or hospital dressings visible by X-ray (see for example WO2015/170569). In one embodiment, the Bi NPs of the invention can be incorporated in different medical
10 devices and implantable medical materials (for example: catheters, endotracheal tubes, hydrogel, wound dressings, bath wipes, hospital textiles, respirators, surgical meshes, breathing masks, vascular grafts, ventricular assist devices, bone and orthopedic cements, implants for joint replacement, bone-substitute materials, dental materials, composite resins, opaque ribbons for surgical sponge markers, inks, beads for embolization) to give
15 them an X ray radioopacity. The Bi NPs of the invention can also be incorporated in polymers or plastic to give them an X ray radioopacity (radiopaque polymeric materials used as medical implants or inserts).

In one embodiment, the Bi NPs of the invention are useful as X-ray radiosensitizers. In a preferred embodiment, the Bi NPs of the invention are useful as X-ray radiosensitizers in
20 radiotherapy or in photothermal therapy.

In one embodiment, the Bi NPs of the invention are useful in radiotherapy.

In one embodiment, the Bi NPs of the invention are useful in photothermal therapy by infra-red.

The invention also relates to a medicament comprising the Bi NPs of the invention as
25 active principle. In one embodiment, the Bi NPs of the invention are for use as medicament. In one embodiment, the invention relates to the use of the Bi NPs of the invention for the manufacturing of a medicament. The invention thus provides methods of treatment and/or prevention of diseases, comprising the administration of a

therapeutically effective amount of the Bi NPs of the invention, to a patient in need thereof.

Depending on the bioactive group present on the Bi NPs, a broad variety of diseases may be targeted. In a specific embodiment, the Bi NPs of the invention are useful as anticancer
5 agents. In a specific embodiment, the Bi NPs of the invention are useful as anti-inflammatory agents.

According to one embodiment, the Bi NPs of the invention may be administered as part of a combination therapy. Thus, are included within the scope of the present invention
10 embodiments comprising co-administration of the Bi NPs of the present invention as active ingredient and additional therapeutic agents and/or active ingredients.

In one embodiment, the Bi NPs of the invention are also useful as biocide agents. By “biocide agent” is referred to a compound that has bactericide, antimicrobial, antibacterial, antimycotical, fungicide, antiparasitic, antibiofilm, antibiofouling, antiviral properties.

15 For this type of indications, many formulations of the Bi NPs are possible, such as for example liquids, gels, suspensions, micelles, emulsions, submicrons colloidal systems (liposomes, nanospheres, nanocapsules) cements, resins fibers, and also different technology of coating of Bi NPs on devices can be used as described for example in WO2006/026026.

20 When the Bi NPs of the invention are used as bactericide agents, they may be used for example in plasters, catheters, clothes, antibacterial packaging, food washing product, surface disinfectant, water disinfectant, air disinfectant (for example in air conditioning system), refrigeration, dental products, bone cement.

When the Bi NPs of the invention are used as bactericide agents, they may be used as
25 preservative agents for example in cosmetic compositions, or in medical devices such as ophthalmic lenses, catheter, hydrogel, hospital dressing and surgical tools.

In one embodiment, the Bi NPs of the invention are useful in ink compositions, especially in (semi-)conductive ink compositions, suitable for different printing methods such as for example inkjet, spray, serigraphy, rotogravure, flexography, doctor blade, spin coating or slot die coating (see for example WO2015/000796). The invention thus also relates to an
5 ink-composition comprising Bi NPs of the invention. Such ink composition may be useful for example in optoelectronic, photovoltaic or in contactless technologies.

BRIEF DESCRIPTION OF THE DRAWINGS

Characterization of citrate-coated Bi NPs synthesized in batch: **Figure 1** is a graph
10 representing the zeta potential in function of pH; **Figure 2** is a UV-Visible spectrum; **Figure 3** is an IR spectrum; **Figure 4** is a TGA spectrum; **Figure 5** is an XRD diagram; **Figure 6** is an XPS diagram of 4f electron; and **Figure 7** is a TEM histogram.

Characterization of citrate-coated Bi NPs synthesized in continuous flow under microwave activation: **Figure 8** is an IR spectrum; **Figure 9** is an UV-visible spectrum;
15 **Figure 10-1** is a TEM histogram; **Figure 10-2** is an XPS diagram of 4f electron; **Figure 10-3** is a TGA spectrum.

Characterization of citrate-coated Bi NPs synthesized in continuous flow under thermal activation: **Figure 11** is an IR spectrum; **Figure 12-1** is an UV-visible spectrum; **Figure 12-2** is an XPS diagram of 4f electron; **Figure 12-3** is a TGA spectrum and
20 **Figure 13** is a graph representing the evolution overtime of the absorbance at pH 7.

Characterization of lipoic acid-coated Bi NPs synthesized in continuous flow under thermal activation: **Figure 14** is a UV-visible spectrum.

Characterization of lipoic acid-coated Bi NPs synthesized by coating exchange at room temperature: **Figure 15** is an IR spectrum; **Figure 16** is an UV-visible spectrum; and
25 **Figure 17** is a graph representing the potential zeta in function of pH.

Characterization of lipoic acid-coated Bi NPs synthesized by coating exchange under microwave irradiation: **Figure 18** is an IR spectrum.

Characterization of lipoic acid-octadecylamine coated Bi NPs synthesized by coating exchange: **Figure 19-1** is an IR spectrum and **Figure 19-2** is a TGA spectrum.

Characterization of tetraethoxysilane coated Bi NPs synthesized by silanization: **Figure 20** is an IR spectrum and **Figure 21** is an UV-visible spectrum.

- 5 Characterization of 3-aminopropyltriethoxysilane coated Bi NPs synthesized by silanization: **Figure 22** is an IR spectrum and **Figure 23** is an UV-visible spectrum.

Characterization of 3-(propyltrimethoxysilane)methacrylate coated Bi NPs synthesized by silanization: **Figure 24** is an IR spectrum.

10 EXAMPLES

The present invention is further illustrated by the following examples.

Material and Methods

- Bismuth citrate (Alfa Aesar, >94%), D-glucose (Sigma, 99.5%), citric acid (Labosi, >98%), lipoic acid (TCI, >97%), bismuth chloride (Acros, >99%), bismuth subnitrate
15 (Aldrich), bismuth acetate (Alfa Aesar, >99%), bismuth citrate ammonium (Fluka >99%), meso-2,3-dimercaptosuccinic acid (TCI, >95%), dithizone (TCI, >85%), cysteine (TCI, >98%), acetyl cysteine (TCI, >98%), sodium hexametaphosphate (Alfa Aesar), glucosamine (Aldrich, >99%), diethylenetriamine (Aldrich, >97%), cyclodextrine (Fluka, >95%), tetraethoxysilane (Alfa Aesar, 98%), 3-propylaminotriethoxysilane (Fluka, 95%),
20 2-[3-(triethoxysilyl)propyl]succinic acid (ABCR, 94%), (3-trimethoxysilyl)propyl methacrylate (TCI, >98%), 2,2,1,1-tridecafluoro-*n*-octylsilane (TCI, >98%), glutathion (TCI, >97%), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Alfa Aesar, 98%), hydroxybenzotriazole (Aldrich, >97%).

- Centrifugation was performed with a Biofuge Primo (Instrument Heraeus).
25 Lyophilization was performed using a HETO powerdry LL3000 lyophilizer (ThermoFisher). Measurements and pH adjustments were performed by automatic

titration on a 702SM (Metrohm) titrimeter with a combined glass electrode. The microwave system is a CEM Discover Microwave Synthesizer (CEM Corporation, USA).

Dynamic light scattering (DLS) and zeta potential measurements were performed with a ZS90 Nano Series ZetaSizer (Malvern Instrument) equipped with a 633 nm wavelength
5 laser and 175° scattering angle. The analyzed solutions were prepared at 1 mM. Infrared spectroscopy was performed on a Perkin Elmer Spectrometer. The spectra were recorded with a resolution of 4 cm⁻¹ and 64 scans. Absorbance measurements for UV-visible spectroscopy (UV-Visible) were made on a Genesys 10UV Scanning UV spectrometer (Thermo spectronic). Thermogravimetry (TGA), i.e. measurements of thermal stability
10 and composition analysis, were carried out on a TGA Q500 type measuring instrument (TA instruments, USA) under a nitrogen flow of 50 ml/min. The temperature programmed to go from 25°C to 400°C with a temperature gradient of 10°C/min. X-ray diffraction (XRD) experiments were performed on a D5000 Siemens diffractometer using the Cu K α radiation ($\lambda=0.15406$ nm). The scattering intensities were measured over an
15 angular range of 20°<2 θ <80° for all samples. One measurement was recorded every 0.05° during 25 seconds. The measurements were recorded at room temperature. X photoelectron spectroscopy (XPS) measurements were made on a Phi Versa 5000 XPS spectrometer. The images of transmission electron microscopy (TEM) were taken on a Fei Tecnai 10 microscope operating at 80 kV (Oregon, USA). The samples were prepared
20 by placing a drop of a dilute suspension of bismuth nanoparticles on a carbon-coated copper grid (300 mesh) or on a copper grid.

Example 1a: Comparative example

Purpose: Reproduction of Example 1 of Chinese Patent CN 104668578 A.

Protocol: 0.97 g of bismuth nitrate and 2.98 g of glucose are mixed in 100 ml of water at
25 60°C at 300 rpm for 2 hours. The suspension is then mixed at 25°C for 12 h and then 2 g of NaOH are added in 40 ml of water with stirring at 60°C. Then, a solution of NaOH is added dropwise to the solution containing the bismuth under ultrasound at 80°C. Then the mixture is agitated during 2h. The solution is washed with ethanol, water and acetone by centrifugation.

Results: Reagents are never soluble. In addition, the bismuth nanoparticles are not stable at the end of production, as shown by their immediate precipitation. The bismuth nanoparticles sizes measured in DLS after centrifugation are as follows (Table 1):

Table 1. Size (DLS) of the Bi NPs of the Chinese patent, in intensity and in number.

Size (DLS) in intensity	Size (DLS) in number
886 $\pm$ 201 (99%)	836 $\pm$ 210 (100%)

- 5 Conclusion: Using the protocol described in Chinese Patent CN 104668578 A, it is concluded that:
- the bismuth nitrate salt, used as bismuth precursor, is not soluble: these conditions are consequently not compatible with continuous flow synthesis;
 - the sizes of the nanoparticles are very large and polydisperse;
 - 10 – the nanoparticles obtained by this process are not stable (precipitation is observed immediately after the synthesis).

Example 1b: Comparative example

Purpose: Determining the nature of the coating of the nanoparticles disclosed by Hernandez-Delgadillo et al. in International Journal of Nanomedicine, Vol. 7, 2012, pp.
15 2109-2113.

Protocol: 0.0148 g of $\text{Na}_3(\text{C}_6\text{H}_5\text{O}_7) \cdot 2\text{H}_2\text{O}$ were dissolved in 100 μL of water and 24 mL of dimethyl sulfoxide (DMSO) was added. Next, 0.0242 g of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ were added to the mixture. The mixture was fizzed with argon for 15 minutes, and 1 mL of sodium borohydride in methanol 0.2 M NaBH_4 was added as reducing agent.

- 20 The resulting nanoparticles were analyzed by infrared spectroscopy: a large bond around 1017 cm^{-1} was observed, corresponding to $\text{S}=\text{O}$ and as well as a bond around 1407 and 1415 cm^{-1} , corresponding to $\text{C}-\text{S}$. Bonds around 2915 cm^{-1} and 2998 cm^{-1} were also observed, corresponding to $\text{C}_{\text{sp}^3}-\text{H}$.

Conclusion: In the nanoparticles of Hernandez-Delgadillo et al., the stabilizing coating
25 agent is not citric acid or citrate but it is dimethyl sulfoxide.

Example 2: Selection of water-soluble bismuth (III) precursors

Purpose: Selection of water-soluble salts of bismuth allowing the formation of Bi NPs in particular in continuous flow synthesis.

Protocol: The bismuth salt (0.01 M, 1 eq.) and glucose (0.017 M, 1.7 eq., 16 mg) are
 5 solubilized in a solution of NaOH (5 ml, 1 M). The pH is measured and the reaction is carried out under microwave irradiation at a power of 75 W and at a temperature of 120°C for 2 minutes and 10 seconds. At the end of the reaction, the pH is again controlled. The reaction medium is acidified to pH 10 with a nitric acid solution (1 M). Ultrafiltration is performed with a 5 kDa cellulose filter (Merck). A washing solution at pH 10 allows to
 10 purify the Bi NPs. The ultrafiltration (UF) is complete when the conductivity value of the filtrate is identical to value of the wash water (approximately 50 μ S/cm). The formation of nanoparticles is controlled by pH variation and visual aspect (Table 2).

Table 2. Solubility of bismuth precursors and formation of Bi NPs.

Complexing salt of bismuth (III)	Solubility at pH 14	Initial pH	Final pH	Theoretical pH	pH jump	Formation of Bi NPs
chloride	No	13.71	13.70	13.66	No	No
acetate	No	13.69	13.68	16.65	No	No
nitrate	No	13.68	13.67	13.64	No	No
citrate	Yes	13.68	13.64	13.64	Yes	Yes
ammonium citrate	Yes	13.76	13.72	13.73	Yes	Yes
lipoic acid	Yes	13.64	13.60	13.60	Yes	Yes
dimercaptosuccinic acid	Yes	13.65	13.61	13.61	Yes	Yes

Conclusion: The choice of the bismuth salt precursor depends of (1) its solubility, for a
 15 homogeneous synthesis; and (2) the achievement of a complete pH jump for a complete conversion in bismuth metallic nanoparticles. Citrate, ammonium citrate, lipoic acid, dimercaptosuccinic acid are bismuth precursors fulfilling these 2 criteria.

Example 3: Comparison of a microwave versus thermal activation

Purpose: Compare microwave versus thermal activation for the formation of Bi NPs.

20 Protocol 1 for thermal activation: The Bi NPs are synthesized in batch by thermal heating at 80°C or 120°C. Bismuth citrate (0.01 M, 1 eq., 20 mg) with glucose (0.017 mM,

1.7 eq., 16 mg) are solubilized in a solution of NaOH (5 ml, 1 M) without stirring at 120°C for 2h30 or 80°C during 3h30. At the end of the reaction the pH jump is reached and Bi NPs are observed. The reaction medium is acidified to pH 10 using nitric acid (1 M). The Bi NPs are isolated after 3 washes in centrifugation at a speed of 4000 rpm.

5 The first wash lasts 45 minutes and the other two 30 minutes.

Protocol 2 for microwave activation: The Bi NPs are synthesized in batch under microwave irradiation at 75 W according to the protocol. Bismuth citrate (0.01 M, 1 eq, 20 mg) with glucose (0.017 mM, 1.7 eq., 16 mg) are solubilized in a solution of NaOH (5 ml, 1 M) at 75 W for 2 minutes 10 seconds. At the end of the reaction the pH jump is reached and Bi NPs are observed. The reaction medium is acidified to pH 10 using nitric acid (1 M). The Bi NPs are isolated after 3 washes in centrifugation at a speed of 4000 rpm The first wash lasts 45 minutes and the other two 30 minutes.

Table 3. Size and population distribution (measured by DLS) of citrate-coated Bi NPs synthesized under microwave or thermal activation.

Activation	DLS size (nm) and population distribution (percentage)	Synthesis time
Thermal at 120°C	94±22 (64%)	2h30
	222±89 (35 %)	
Thermal at 80°C	350± 70 (15 %)	3h30
	1277± 754 (85%)	
Microwave (120°C at 75W)	115±34 (82%)	2min 10
	315±87 (17%)	

15 Conclusion: Irradiation under microwave allows to obtain more rapidly Bi NPs.

Example 4: Reproducible synthesis of Bi NPs in batch

Purpose: Provide reproducible protocols for batch synthesis of Bi NPs.

Protocol 1 (pH 14): The nanoparticles are synthesized in batch under microwave irradiation at pH 14. Bismuth citrate (0.01 M, 1 eq., 20 mg) and glucose (0.017 M, 1.7 eq., 16 mg) are solubilized in a solution of NaOH (1 M, 5 ml). The pH is measured and the reaction is carried out under microwave (MW) irradiation at a power of 75 W and at a temperature of 120°C for 2 minutes and 10 seconds. The end of the reaction, the pH

is again controlled and a jump of pH is observed. The reaction medium is acidified to pH 10 with a nitric acid solution (1 M). Ultrafiltration is performed on a 5 kDa cellulose filter (Merck). A washing solution at pH 10 allows to purify the bismuth nanoparticles. The conductivity value of the filtrate is identical to value of the wash water
5 (approximately 50 $\mu\text{S}/\text{cm}$).

Table 4. Size (measure by DLS) of citrate-coated Bi NPs synthesized in batch under microwaves irradiation at pH 14.

Operator	Initial pH	Number of experiments	Size (nm) and population distribution (percentage) measured by DLS
A	14	9	113 $\pm$ 40 (100%)
B	14	2	127 $\pm$ 30 (100%)

Protocol 2 (pH 13): The nanoparticles are synthesized in batch under microwave irradiation at pH 13. Bismuth citrate (0.01 M, 1 eq., 20 mg) and glucose (0.017 M,
10 1.7 eq., 16 mg) are solubilized in a solution of NaOH (0.1 M, 5 ml). The pH is measured and the reaction is carried out under MW irradiation at a power of 75 W and a temperature set point of 140°C for 2 minutes and 30 seconds. At the end of the reaction, the pH is again controlled and a pH jump is observed. The reaction medium is acidified to pH 10 with a nitric acid solution (1 M). Ultrafiltration is performed on a 5 kDa cellulose filter
15 (Merck). A washing solution at pH 10 allows to purify the bismuth nanoparticles. The UF is complete when the conductivity value of the filtrate is identical to value of the wash water (approximately 50 $\mu\text{S}/\text{cm}$).

Table 5. Size (measured by DLS) of citrate-coated Bi NPs synthesized in batch under microwaves irradiation at pH 13.

Experiment	Initial pH	Size (nm) and population distribution (percentage) measured by DLS
1	13	130 $\pm$ 120 (100%)
2	13	125 $\pm$ 120 (100%)
3	13	140 $\pm$ 105 (100%)

20 Conclusion: Bi NPs syntheses with the process of the invention are reproducible either at pH 14 or at pH 13.

Example 5: Metallic bismuth nanoparticles synthesis yield

Purpose: Assess the yield of bismuth metallic nanoparticles synthesis.

Protocol: The amount of bismuth (III) is determined by UV-visible spectrometry after digestion of the nanoparticles, by complexing Bi (III) by dithizone. The peak of absorbance is at 493 nm.

Operating mode of the UV-visible calibration line. Bismuth (20mg) is solubilized in HCl solution (0.1 M, 1 L). Several volumes 0.7; 0.9; 1; 1.15; 1.25 and 1.5 ml of the bismuth solution are removed and then these solutions are completed with 1 M HCl solution (up to 1 mL) to obtain volume of 1.50 ml. Acetate buffer pH 4 (1 ml) and a freshly prepared solution of dithizone 10% (w/v) solubilized in ethanol (1 ml) are added. To reach a total volume of 10 ml, distilled water (6.5 ml) are added. The white contains a solution of HCl (0.1 M, 1 ml), a solution of ethanol (1 ml) and is made up to 10 ml with distilled water.

Synthesis of bismuth nanoparticles. The nanoparticles are synthesized in batch under microwave irradiation at 75 W according to protocol 1 of example 4.

Digestion and evaluation of bismuth yield. Bismuth nanoparticles (2 mg) are solubilized in a solution of hydrochloric acid (1 M, 5 ml). The medium is submitted to MW irradiation for 2 hours at 100°C. 90 ml of distilled water are dissolved in order to obtain a solution of HCl (0.1 M, 100 ml). This solution makes it possible to carry out a measurement in UV-visible spectroscopy.

Note: The acid solution should be diluted because dithizone is sensitive to sodium concentration.

Table 6. Yield of citrate-coated Bi NPs synthesized in batch under microwaves irradiation at pH 14.

Experiment	Yield of synthesis in bismuth %
1	94%
2	98%
3	96%

Conclusion: The three results are concordant. The yield of bismuth in the nanoparticles is greater than 94%. This result is consistent with the final pH measurements that shows the total reduction of the Bi (III) salt to Bi (0) to form the nanoparticles.

Example 6: Characterizations of Bi NPs synthesized in batch

5 Purpose: Characterize metallic bismuth nanoparticles (0) synthesized in batch.

Protocol: The nanoparticles are synthesized in batch under microwave irradiation according to protocol 1 of example 4. The nanoparticles are analyzed according to several techniques as detailed below.

Zeta Potential: The zeta potential of the Bi NPs is measured between pH 7 and pH 13.

10 Zeta potential is less than 30 mV between pH 9 and pH 11, as shown on Figure 1.

UV-Visible Spectroscopy: The UV-Visible spectrum (Figure 2) of the nanoparticles is identical to the UV spectrum described in the literature (M Port, G Hallot, C Gomez. Inorganic frameworks as Smart Nanomedicines 1st edition. A publisher Grumezescu. Elsevier 2018). Absorbance across the spectral domain shows that the nanoparticles of
15 bismuth are black. The peak at 275 nm is a specific absorbance of citrate-coated metal bismuth nanoparticles.

Infrared Spectroscopy: The infrared spectrum (Figure 3) of the nanoparticles shows a bond at 3239 cm^{-1} corresponding to the vibration of the hydroxyl bond. The bond at 2856 cm^{-1} corresponds to the vibration of the $\text{C}_{\text{sp}^3}\text{-H}$ bond. The bond at 1554 cm^{-1} and
20 1380 cm^{-1} for symmetric and asymmetric stretching vibrations of the C=O bond of carboxylates. The IR spectrum allows to identify the coating agent as a hydroxy acid.

Thermogravimetry (TGA): The TGA spectrum (Figure 4) shows a loss of water between 50°C and 100°C , and a second loss at 250°C corresponding to citrate. There are 3 citrates per nm^2 on the surface of the nanoparticles.

25 X-ray Diffraction (XRD): The XRD diagram (Figure 5) shows the presence of metallic bismuth by the presence of crystalline planes (012), (104) and (110) corresponding to

literature description (Brown et al., Chemistry of Materials, Volume 26, 2014, pages 2266-2274).

X photoelectron spectroscopy (XPS): The XPS diagram (Figure 6) shows the presence of a 75% surface area of bismuth oxide and 25% of metallic bismuth at the surface of the
5 bismuth nanoparticles.

Transmission electron microscopy (TEM): The size of nanoparticles in TEM is on average of 12 nm as shown on the TEM histogram (Figure 7).

Dynamic light scattering (DLS): The mean size of the nanoparticles in DLS is on average of 120 nm (see example 4).

10 Conclusion: All the analyses demonstrate the achievement of metallic bismuth nanoparticles by the synthesis in batch.

Example 7: Synthesis of Bi NPs in continuous flow under microwave activation from bismuth citrate

Purpose: Synthesize bismuth metallic nanoparticles in continuous flow under microwave
15 activation from bismuth citrate.

Protocol for continuous flow under microwave irradiation with valve 1° (conicity 1°): In a conical flask, a quantity of citric acid (0.04 M, 630 mg, 10 eq.) is dissolved in distilled water (76 ml). The pH is adjusted with an aqueous solution of NaOH (5 M) in order to obtain a pH equal to 13.1 and a total volume of 80 ml. Bismuth citrate (0.004 M, 120 mg,
20 1 eq.) and glucose (0.015 M, 192 mg, 3 eq.) are added and solubilized to the previous solution. The pH is rechecked and must be equal to 13. The solution is then pumped by an HPLC pump at a flow rate of 0.75 ml/min and then irradiated in the microwave with a power of 25 W and a set temperature of 140°C. The tubing in which the solution passes and which is maintained by a Teflon support. The flow output, a flow controller (valve 1
25 conicity 1°) is installed and set to a flow rate of 1 turn. The bismuth nanoparticles are isolated by ultrafiltration on a 30 kDa filter (Merck) and washed with water at pH 7.

Protocol for continuous flow under microwave irradiation with valve 3 (conicity 3°): In a conical flask, a quantity of citric acid (0.04 M, 630 mg, 10 eq.) is dissolved in distilled water (76 ml). The pH is adjusted with an aqueous solution of NaOH (5 M) in order to obtain a pH equal to 13.1 and a total volume of 80 ml. Bismuth citrate (0.004 M, 120 mg, 1 eq.) and glucose (0.013 M, 162 mg, 3 eq.) are added and solubilized to the previous solution. The solution is then pumped by an HPLC pump at a flow rate of 1.3 ml/min and then irradiated under microwave with a power of 25 W and a set temperature of 140°C the tubing in which the solution passes and which is maintained by a Teflon support. The flow output, a flow controller (valve 3: conicity 3°) is installed and set to a flow rate of 1 turn. The bismuth nanoparticles are isolated by ultrafiltration on a 30 kDa filter (Merck) and washed with water at pH 7.

DLS and reproducibility (Table 7): The size of Bi NPs was measured by DLS.

Table 7: Size (measured by DLS) of Bi NPs synthesized in continuous flow under microwave irradiation.

Experiment	Valve	DLS size (nm) and population distribution (percentage)
1	3	58± 19 (100%)
2		49± 18 (100%)
3		51 ±28 (100%)
4	1	52± 19 (100%)

Infrared spectroscopy: The infrared spectrum (Figure 8) of the nanoparticles shows bond at 3409 cm⁻¹ corresponding to the vibration of the hydroxyl bond. The bond at 1554 cm⁻¹ and 1380 cm⁻¹ for symmetric and asymmetric stretching vibrations of the C=O bond of carboxylates. The IR spectrum allows to identify the coating agent as a hydroxy acid.

UV-Visible spectroscopy: In the UV-Visible spectrum (Figure 9), absorbance across the spectral range shows that the nanoparticles of bismuth are black. The peak at 275 nm is a specific absorbance of citrate coated bismuth nanoparticles.

Transmission electron microscopy (TEM): The nanoparticles obtained in continuous flow under microwave irradiation measure 2 nm according to TEM images (Figure 10-1).

X photoelectron spectroscopy (XPS): The XPS diagram (Figure 10-2) shows the presence of a 100% surface area of bismuth oxide at the surface of the bismuth nanoparticles.

Thermogravimetry (TGA): The TGA spectrum (Figure 10-3) shows a loss of water between 50°C and 100°C, and a second loss at 250°C corresponding to citrate. There is
5 one citrate per nm² on the surface of the nanoparticles.

Conclusion: All the analysis demonstrates the achievement of metallic bismuth nanoparticles by in continuous flow under microwave activation from bismuth citrate. The synthesis is reproducible.

10 **Example 8: Synthesis of Bi NPs in continuous flow under thermal activation from bismuth citrate**

Purpose: Synthetize Bi NPs in continuous flow under thermal activation from bismuth citrate.

Protocol for continuous flow under thermal activation with valve 1° (conicity 1°): In a conical flask, a quantity of citric acid (0.004 M, 630 mg, 10 eq.) is dissolved in distilled
15 water (76 ml). The pH is adjusted with an aqueous solution of NaOH (5 M) in order to obtain a pH equal to 13.1 and a total volume of 80 ml. Bismuth citrate (0.004 M, 120 mg, 1 eq.) and glucose (0.013 M, 162 mg, 3 eq.) are added and solubilized to the previous solution. The pH is rechecked and must be equal to 13. The solution is then pumped by an HPLC pump at a flow rate of 0.75 ml/min is placed in a water bath heated to 95°C
20 where is placed the tubing in which passes the solution and which is maintained by a Teflon support. The flow output, a flow controller (valve 1) is installed and set to a flow rate of 1 turn. The bismuth nanoparticles are isolated by ultrafiltration on a 30 kDa filter (Merck) and washed with water at pH 7.

Protocol for continuous flow under thermal activation with valve 3° (conicity 3°): In a
25 conical flask, a quantity of citric acid (0.04 M, 630 mg, 10 eq.) is dissolved in distilled water (76 ml). The pH is adjusted with an aqueous solution of NaOH (5 M) in order to obtain a pH equal to 13.1 and a total volume of 80 ml. Bismuth citrate (0.004 M, 120 mg, 1 eq.) and glucose (0.013 M, 162 mg, 3 eq.) are added and solubilized to the previous

solution. The pH is rechecked and must be equal to 13. The solution is then pumped by an HPLC pump at a flow rate of 1.5 ml/min is placed in a water bath heated to 95°C where is placed the tubing in which passes the solution and which is maintained by a Teflon support. The flow output, a flow controller (valve 3) is installed and set to a flow rate of 1 turn. The bismuth nanoparticles are isolated by ultrafiltration on a 30 kDa filter (Merck) and washed with water at pH 7.

DLS and reproducibility (Table 8): The size of Bi NPs was measured by DLS.

Table 8: Size (measured by DLS) of citrate-coated Bi NPs synthesized in continuous flow under thermal activation.

Test	Valve	DLS size (nm) and population distribution (percentage)
1	1	34± 15 (100%)
2		35± 15 (100%)
3		34± 19 (100%)
4	3	54±26 (100%)
5		55±17 (100%)
6		53±16 (100%)

Infrared spectroscopy: The infrared spectrum (Figure 11) of the nanoparticles shows bonds 1575 cm⁻¹ and 1350 cm⁻¹ for the symmetric stretching of the C=O and C-O bonds of the carboxylates. The bond at 3309 cm⁻¹ corresponds to the vibration of the hydroxide bond.

UV-visible spectroscopy: In the UV-Visible spectrum (Figure 12-1), absorbance across the spectrum shows that the nanoparticles of bismuth are black. The peak at 275 nm is a specific absorbance of citrate coated bismuth nanoparticles.

X photoelectron spectroscopy (XPS): The XPS diagram (Figure 12-2) shows the presence of a 100% surface area of bismuth oxide at the surface of the bismuth nanoparticles.

Thermogravimetry (TGA): The TGA spectrum (Figure 12-3) shows a loss of water between 50°C and 100°C, and a second loss at 250°C corresponding to citrate. There are 3 citrate per nm² on the surface of the nanoparticles.

Transmission electron microscopy (TEM): The size of nanoparticles in TEM is on average of 3.6 nm.

Degradation at pH 7: The degradation was studied using the following protocol. The pH of a nanoparticle solution (1 mM) is adjusted to pH 7, this solution is monitored daily by
5 UV-visible spectroscopy for the evolution of absorbance and in DLS for measurements of size and stability. No degradation is observed in UV-visible over 50 days (Figure 13). DLS analysis shows that bismuth metallic nanoparticles are stable at least for 24 days at pH 7.

Conclusion: All the analysis demonstrate the achievement of bismuth metallic
10 nanoparticles when synthesized in continuous flow under thermal activation from bismuth citrate. The synthesis is reproducible. The metallic bismuth nanoparticles are stable at pH 7 over 24 days.

Example 9: Synthesis of Bi NPs in continuous flow with thermal activation from bismuth lipoate

15 Purpose: Synthesize Bi NPs in continuous flow under thermal activation from bismuth lipoate.

Protocol: The nanoparticles are synthesized in continuous flow under thermal activation. Bismuth chloride (0.004 M, 1 eq., 95 mg) is solubilized in water (76 ml) and then lipoic acid (0.016 M, 4 eq., 250 mg) is added. After solubilization, the solution is transferred to
20 a conical flask. The pH of the solution is adjusted with an aqueous solution of NaOH (5 M) to obtain a pH equal to 13.1 and a total volume of 80 ml. The glucose (0.013 M, 162 mg, 3 eq.) is added and solubilized to the previous solution. The pH is rechecked and must be equal to 13. The solution is then pumped by an HPLC pump at a flow rate of 0.75 ml/min is placed in a water bath heated to 95°C where is placed the tubing in which
25 passes the solution and which is maintained by a Teflon support. The flow output, a flow controller (valve 3) is installed and set to a flow rate of 1 turn.

DLS, zeta potential and stability at pH 13 (Table 9): The measurements were carried out at pH 13.

Table 9: Size (measured by DLS) of lipoic acid-coated Bi NPs synthesized in continuous flow under microwave irradiation.

DLS size (nm) and population distribution (percentage) at pH 13	Zeta at pH 13	Stability at pH 13
89±39 100%	-48 mV	at least 3h

UV-visible spectroscopy: In the UV-Visible spectrum (Figure 14), an absorption peak at 400 nm is observed by UV-visible spectroscopy characteristic of bismuth nanoparticles with thiol coating.

Conclusion: Lipoic acid-coated Bi NPs are synthesized in continuous flow under microwave irradiation and are stable at least 3h at pH 13.

Example 10: Coating exchange – Modification of the coatings of Bi NPs activated by microwave irradiation

10 Purpose: Modify the coating of Bi NPs by coating exchange activated by microwave irradiation.

The nanoparticles are synthesized in batch under microwave irradiation. Bismuth citrate (0.01 M, 1 eq., 20 mg) and glucose (0.017 M, 1.7 eq., 16 mg) are solubilized in a solution of NaOH (5 ml, 1 M). The pH is measured and the reaction is carried out under MW
15 irradiation at a power of 75 W and a temperature set point of 120°C for 2 minutes and 10 seconds. At the end of the reaction, the pH is again controlled and a pH jump is observed.

Coating exchange: The coating agent (5 eq.) is added to the previous solution and the pH is readjusted according to the pH of the previous solution. The solution is then activated
20 at 75 W for 1 min. The nanoparticles are isolated by centrifugation at 4000 rpm for 45 min with distilled water.

Stability assessment: A nanoparticle solution (1 mM) is prepared in a pillbox. The stability with regards to agglomeration and/or aggregation and/or precipitation is assessed by a visual test (appearance of a black precipitate). The samples remained stable to
25 precipitation for at least 2 hours.

Results: The exchanges of the coating are followed by measuring the size in DLS, the stability of the nanoparticles and zeta potential at pH 7 and pH 10 (Table 10).

Table 10: Different coating exchange under microwave irradiation.

Coating agents	Zeta potential at pH 7 (mV)	Zeta potential at pH 10 (mV)	DLS size (nm) & population distribution (percentage) pH 7	DLS size (nm) & population distribution (percentage) pH 10
Lipoic acid	-35	-35	342± 107 (72%) 4429± 910 (1%) 136± 19 (27%)	385± 97 (76%) 155 ±28 (24%)
Mercapto succinic acid	-26	-40	655 ±2 74 (100%)	91± 12 (28%) 155± 67 (67%) 491± 119 (3%)
Cysteine	-33	-41	67± 15 95% 263± 125 5%	48± 10 30% 202± 86 70%
Acetyl cysteine	-26	-33	52± 11 60% 150± 70 40%	55± 16 35% 178± 76 66%
Sodium hexametaphosphate	-35	-49	86± 15 (63%) 292± 66 (30%) 790± 147 (8%)	256± 41 (25%) 650± 204 (71%) 3351± 785 2%
Glucosamine	-38	-49	197±51 (25%) 681± 143 (12%) 73± 10 (63%)	104± 16 (60%) 327± 87 (40%)
Diethylenetriamine	-35	-35	303± 163 (100%)	590± 177 (9%) 175±39 (91%)
Cyclodextrine	-37	-41	64± 9 (35%) 110± 26 (60%) 370± 95 (5%)	658± 158 (4%) 103± 22 (96%)
Pectine	-39	-44	575± 105 (100%)	341± 75 (41%) 901± 305 (56%) 4515± 879 (3%)

Conclusion: Coating exchange by microwave activation is efficient with different coating agents.

Example 11: Coating exchange – Modification of Bi NPs at room temperature with lipoic acid

Purpose: Modify the coating of Bi NPs by coating exchange with lipoic acid at room temperature.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The modification of the surface of the nanoparticles of bismuth is then carried out by adding lipoic acid (2 eq., 15 mg) to a solution of nanoparticles (5 ml, 3 mM) at pH 10. The solution is stirred for 24 h at room temperature.

DLS and zeta potential (Table 11): The size of Bi NPs was measured by DLS and zeta potential was also determined at pH 6.5.

Table 11: Size (measured by DLS) of lipoic acid-coated Bi NPs synthesized by coating exchange at room temperature.

DLS size (nm) and population distribution (percentage)	Zeta potential at pH 6.5
19 ± 3 (32%)	-15 mV
58 ± 26 (68%)	

Infrared spectroscopy: In the infrared spectrum (Figure 15), the bond at 3350 cm⁻¹ corresponds to OH and the bond at 2925 cm⁻¹ corresponds to the vibration mode of the Csp³-H bond. The two bonds 1575 cm⁻¹ and 1325 cm⁻¹ correspond to C=O and C-O.

UV-visible spectroscopy: In the UV-Visible spectrum (Figure 16), absorbance across the spectral range shows that the nanoparticles of bismuth are black. The peak at 330 nm is a specific absorbance of nanoparticles of bismuth coated with lipoic acid.

Zeta potential as a function of pH: As shown on the graph of Figure 17, the zeta potential is of -30 mV above at pH 9.

Conclusion: Coating exchange with lipoic acid at room temperature is efficient. The presence of lipoic acid on the surface of bismuth metallic nanoparticles is demonstrated by above characterizations.

Example 12: Coating exchange – Modification of Bi NPs under microwave activation with lipoic acid

Purpose: Modify the coating of Bi NPs by coating exchange with lipoic acid under microwave activation.

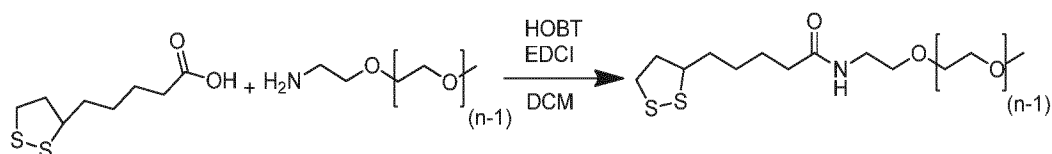
Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The modification of the surface of the nanoparticles of bismuth is carried out by adding lipoic acid (5 eq., 25 mg to a solution of nanoparticles (5 ml, 3 mM) at pH 10. The solution is irradiated under
5 microwave at 75 W for 1 minute.

Infrared spectroscopy: In the infrared spectrum (Figure 18), the bond at 3300 cm^{-1} corresponds to OH and the bond at 2917 cm^{-1} corresponds to the vibration mode of the Csp³-H bond. the two bonds at 1550 cm^{-1} and 1300 cm^{-1} correspond to C=O and C-O.

Conclusion: Coating exchange with lipoic acid under microwave activation is efficient.
The presence of lipoic acid on the surface of bismuth metallic nanoparticles is demonstrated by above characterizations.

Example 13: Synthesis of hydrophilic lipoic coating agents: Lipoic acid-PEG₇₅₀ and Lipoic acid-PEG₁₀₀₀

15 Purpose: Synthesis of coating agents functionalized by a hydrophilic group, namely PEGylated coating agents. PEG₇₅₀-NH₂, PEG₁₀₀₀-NH₂ and PEG₂₀₀₀-NH₂ were used, leading to corresponding PEGylated lipoic acids.



Scheme 1: Coupling reaction between lipoic acid and amino-polyethylene glycol.

Lipoic acid (165 mg, 0.79 mmol, 1 eq.) and PEG-NH₂ (1 eq.) and hydroxybenzotriazole (HOBT) (61.2 mg, 0.4 mmol, 0.5 eq.) are solubilized in anhydrous dichloromethane (26 ml). After solubilization of these reagents, ethyl-3-(3dimethylaminopropyl) carbodiime (EDCI) (575 mg, 2.1 mmol, 3 eq.) is added to the mixture. The reaction is mixed for 48 hours, the solution is treated by acid HCl (1 M, 10 ml) washes and then by NaHCO₃ (10 ml) and by NaCl (10 ml). The organic phase is reconcentrated and then dried. The crude is purified by flash chromatography with a gradient of dichloromethane/methanol (95/5). The isolated product is a yellow oil (0.316 mmol, 0.379g, 40 %).

n= 46 (Lipoic-PEG₂₀₀₀): ¹H-NMR (400 MHz, CDCl₃): δ ppm 3.78-3.46 (m, 180H, 90x CH₂-O), 3.45-3.37 (m, 2H), 3.37 (s, 3H, O-CH₃), 3.2-3.10 (m, 2H), 2.47-2.36 (m, 1H), 2.16 (t, 2H, J= 7.4 Hz), 1.91-1.89 (m, 1H), 1.72-1.55 (m, 4H), 1.49-1.34 (m, 2H).

n= 23 (Lipoic-PEG₁₀₀₀): ¹H-NMR (400 MHz, CDCl₃): δ ppm 3.78-3.46 (m, 90H, 45x CH₂-O), 3.43-3.36 (m, 2H), 3.32 (s, 3H, O-CH₃), 3.17-3.01 (m, 2H, CH₂), 2.46-2.35 (m, 1H, CH₂), 2.18 (t, 2H, J= 7.4 Hz, CH₂), 1.91-1.79 (m, 1H, CH₂), 1.72-1.55 (m, 4H, CH₂), 1.49-1.34 (m, 2H, CH₂).

n = 17 (Lipoic-PEG₇₅₀): ¹H-NMR (400 MHz, CDCl₃): 3.83-3.51 (m, 66H, 33x CH₂-O), 3.32 (s, 3H, O-CH₃), 3.21-3.03 (m, 2H, CH₂), 2.49-2.40 (m, 1H, CH₂), 2.20 (t, 2H, J= 7.2 Hz, CH₂), 1.96-1.84 (m, 1H, CH₂), 1.76-1.69 (m, 4H, CH₂), 1.53-1.39 (m, 2H, CH₂).

Example 14: Coating exchange – Modification of Bi NPs at room temperature with a lipoic-PEG hydrophilic coating agent

Purpose: Modify the coating of Bi NPs by coating exchange with a lipoic-PEG coating agent (from example 13: Lipoic-PEG₇₅₀, Lipoic-PEG₁₀₀₀ and Lipoic-PEG₂₀₀₀).

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The modification of the surface of the nanoparticles of bismuth is then carried out by adding lipoic acid-PEG of example 13 (2 eq., 60 mg) to a solution of nanoparticles (5 ml, 3 mM) at pH 10. The solution is stirred for 24 h at room temperature.

Results: Various parameters were determined for obtained nanoparticles (Table 12):

Table 12: Size (measured by DLS) of lipoic-PEG₇₅₀, lipoic-PEG₁₀₀₀ and lipoic-PEG₂₀₀₀ coated Bi NPs synthesized by coating exchange at room temperature.

	lipoic-PEG ₂₀₀₀	lipoic-PEG ₁₀₀₀	lipoic-PEG ₇₅₀
Size (nm) and population distribution (percentage) at pH 7 (measured by DLS)	71± 53 (100%)	72± 40 (100%)	70± 24 (100%)
Stability of bismuth nanoparticles at pH 7	24 h	30 h	30 h
Zeta potential at pH 7	-18 mV	-15 mV	-18 mV

Size (nm) and population distribution (percentage) in a solution of NaCl 0.9% (nm) (measured by DLS)	81± 43 (100%)	58± 30 (100%)	51± 23 (100%)
Stability of bismuth nanoparticles in a solution of NaCl 0.9%	30 h	48 h	48 h

Further characterizations of lipoic-PEG₇₅₀ coated bismuth metallic nanoparticles:

Infrared spectroscopy: The bond 2870 cm⁻¹ corresponds to the vibration mode of C_{sp3}-H bonds. The two bonds 1548 cm⁻¹ and 1300 cm⁻¹ correspond to C = O and C-O. The bond 1100 cm⁻¹ corresponds to the vibration mode of C-H.

- 5 UV-visible spectroscopy: Absorbance across the spectral range shows that the nanoparticles of bismuth are black. The peak at 330 nm is a specific absorbance of nanoparticles of bismuth coated with lipoic-PEG₇₅₀.

Transmission electron microscopy (TEM): Metallic bismuth nanoparticles obtained in continuous flux under microwave irradiation measure 4 nm according to TEM images.

- 10 Thermogravimetry (TGA): The TGA shows a loss at 350 °C corresponding to lipoic-PEG₇₅₀. There are 3 lipoic PEG₇₅₀ stabilizing groups per nm² on the surface of the nanoparticles.

- Conclusion: The coating exchange is efficient. Notably, lipoic-PEG₇₅₀, lipoic-PEG₁₀₀₀ and lipoic-PEG₂₀₀₀ coated Bi NPs are stable in NaCl 0.9% (representing a physiological media) for at least 30 hours.

Example 15a: Synthesis of a lipophilic lipoic coating agent: lipoic acid-octadecylamine

Purpose: Synthesis of a coating agent functionalized by a lipophilic group (hydrophobic group).



Scheme 2: Coupling reaction between lipoic acid and octadecylamine.

Octadecylamine (1 eq., 250 mg), DMAP (0.25 eq., 26 mg) and DCC (1 eq., 190 mg) are solubilized in DCM (10 ml) under argon at 0°C. In parallel lipoic acid (1 eq., 190 mg) is solubilized in DCM (2.5 ml) and the solution is added to the previous one at 0°C. Then the solution is then stirred for 24h at room temperature. The crude is then filtered through the celite and rinsed with ethyl acetate and the organic phase is washed with water before being dried and concentrated. Column purification was performed to isolate the product in 80% yield (370 mg) DCM/MeOH conditions (99/1). ¹H-NMR (400 MHz, CDCl₃): 3.52 (m, 1H, CH), 3.1 (m, 2H, CH₂) 3.08 (m, 2H, CH₂), 2.3 (m, 1H, CH₂), 2.1 (t, 2H, CH₂), 1.84 (m, 1H, CH₂), 1.62 (m, 4H, CH₂), 1.55 (m, 2H, CH₂), 1.4 (m, 2H, CH₂), 1.2 (m, 30H, CH₂), 0.81 (t, 3H, CH₃).

Example 15b: Coating exchange – Modification of Bi NPs at room temperature with a lipophilic coating agent

Purpose: Modify the coating of Bi NPs by coating exchange with a lipophilic lipoic coating agent (from example 15a: lipoic acid-octadecylamine) and assessment of the solubility in organic solvent and oil of resulting nanoparticles.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The modification of the surface of the nanoparticles of bismuth is then carried out as follows: lipoic acid-octadecylamine of example 15 (8 eq., 45 mg) is solubilized in dichloromethane (10 ml) and is then added to a mixture of water (5 ml) and ethanol (20 ml). The resulting solution is added to a solution of nanoparticles (3 mM, 5 ml) at pH 7. The solution is stirred for 24 hours. Then, the solvents are evaporated.

Infrared spectroscopy: In the infrared spectrum (Figure 19-1), the band at 3350 cm⁻¹ corresponds to the vibration band of the N-H, the band at 2915 and 2830 cm⁻¹ correspond to the mode of vibration of the Csp³-H methyl and CH₂. The bands 1620 and 1480 cm⁻¹ correspond to the carbonyl bands.

DLS size and solubility in different organic solvents and oil (Table 13):

Table 13: DLS size and solubility in different organic solvents and oil.

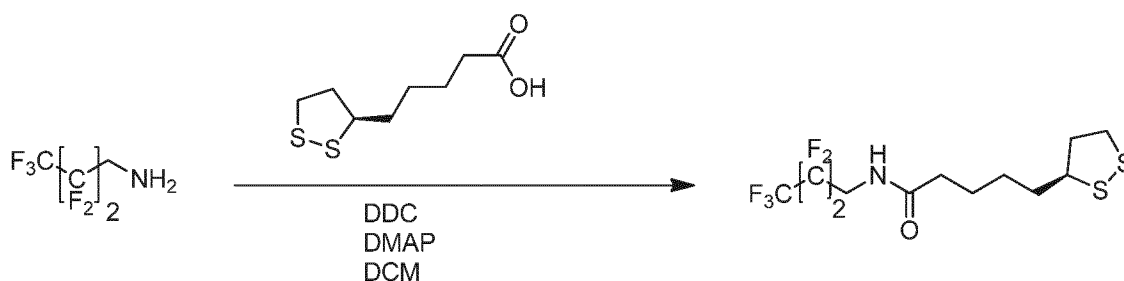
Solvent	Solubility	DLS size (nm) and population distribution (percentage)
Dichloromethane	yes	83± 19 (100%)
Toluene	yes	127± 51 (100%)
Miglyol® 820 oil	yes	214± 45 (100%)

Thermogravimetry (TGA): The TGA spectrum (Figure 19-2) shows a loss at 300°C corresponding to lipoic octadecylamine. There are 6 lipoic octadecylamine stabilizing group per nm² on the surface of the nanoparticles.

- 5 Conclusion: the coating of bismuth metallic was modified by exchange with a lipophilic lipoic compound. Lipophilic metallic bismuth nanoparticles are soluble in organic solvents and oil.

Example 16a: Synthesis of a fluorophilic lipoic coating agent: lipoic acid-heptafluorobutamine

- 10 Purpose: Synthesis of a coating agent functionalized by a fluorophilic group.



Scheme 3 : Coupling reaction between lipoic acid and heptafluorobutamine.

- 2,2,3,3,4,4,4-heptafluorobutamine (1 eq., 250 mg), DMAP (0.25 eq., 26 mg) and DCC (1 eq., 190 mg) are solubilized in DCM (10 ml) under argon at 0 ° C. In parallel lipoic acid (1 eq., 190 mg) is solubilized in DCM (2.5 ml) and the solution is added to the previous one at 0 ° C. Then the solution is then stirred for 24h at room temperature. The crude filter is then filtered through celite and rinsed with ethyl acetate and the organic phase is washed with water before being dried and concentrated. Column purification was performed to isolate the product in 65% yield (370 mg) DCM / MeOH conditions (99/1).
- 20 ¹H-NMR (400 MHz, CDCl₃): δ ppm 5.69 (m, 1H, NH), 4.01 (m, 2H, CH₂), 3.56 (m, 1H, CH),

3.13 (m, 2H, CH₂), 2.45 (m, 1H, CH₂), 2.27 (t, 2H, CH₂), 1.93 (m, 1H, CH₂), 1.69 (m, 4H, CH₂), 1.46 (m, 2H, CH₂).

Example 16b: Coating exchange – Modification of Bi NPs at room temperature with a fluorophilic coating agent

- 5 Purpose: Modify the coating of Bi NPs by coating exchange with a fluorophilic lipoic coating agent (from example 16a: lipoic acid-heptafluorobutamine) and assessment of the solubility in organic solvent and oil of resulting nanoparticles.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The modification of the surface
10 of the nanoparticles of bismuth is then carried out as follows: lipoic acid-heptafluorobutamine of example 16a (8 eq., 45 mg) is solubilized in ethanol (10 ml) and is then added to a mixture of water (5 ml) and ethanol (20 ml). The resulting solution is added to a solution of nanoparticles (3 mM, 5 ml) at pH 7. The solution is stirred for 24 hours. Then, the nanoparticles are purified by ultrafiltration.

- 15 Infrared spectroscopy: In the infrared spectrum, a band at 3322 cm⁻¹ corresponds to the vibration band of the N-H, bands at 2918 and 2850 cm⁻¹ correspond to the mode of vibration of the Csp³-H methyl and CH₂. The bands 1660 and 1548 cm⁻¹ correspond to the carbonyl bands. The bands 1222 and 1115 cm⁻¹ correspond to the C-F bands.

Thermogravimetry (TGA): The TGA spectrum shows a loss at 260°C corresponding to
20 lipoic heptafluorobutamine. There are 4 lipoic heptafluorobutamine stabilizing group per nm² on the surface of the nanoparticles

Table 14: DLS size:

Size (nm) and population distribution (percentage)
44± 13 (100%)
49± 39 (100%)
48± 15 (100%)

Table 15: Solubility in different organic solvents and oil:

Solvent	Solubility
Ethanol	yes
Perfluorooctane bromide	yes

Conclusion: the coating of bismuth metallic was modified by exchange with a perfluorinated lipoic compound. Fluorophilic metallic bismuth nanoparticles are soluble in organic solvents and fluorinated solvents.

5 **Example 17: Silanization by tetraethoxysilane (TEOS)**

Purpose: Coat bismuth metallic nanoparticles with tetraethoxysilane.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. Silanization is then carried out by adding dropwise a solution of TEOS (48 mM, 62 mg, 0.3 mmol, 40 eq.) diluted in ethanol (6 ml) to a aqueous solution of bismuth nanoparticles (1.2 mM, 6 ml, 0.007 mmol, 1 eq.) at pH 10 diluted in absolute ethanol (15 ml). After 24 hours of strong agitation, Bi@SiO₂ nanoparticles are isolated and washed with absolute ethanol by ultrafiltration. The treatment is terminated when the filtrate reaches a conductivity of 0.4 μ S.

Results: Characterizations of silanized particles (Table 16).

15 Table 16: Size and zeta potential of tetraethoxysilane coated Bi NPs obtained by silanization.

Batch	Size DLS (nm) and population distribution (%) (solvent ethanol)	Zeta Potential in ethanol	Zeta Potential in water
1	103 $\pm$ 47 (100%)	-24 mV	- 5.8 mV
2	75 $\pm$ 22 (100%)	-25mV	- 6.82 mV
3	60 $\pm$ 19 100%	-28mV	- 6.5 mV
4	80 $\pm$ 26 (100%)	-27mV	-6 mV

Infrared spectroscopy: In the infrared spectrum (Figure 20), the disappearance of the vibration bands OH, C=O and C-O prove the separation of the citrate in favor of the coating of silica. The increase of the band to 1000 cm⁻¹ corresponds to the Si-O-Si bond. The 1500 cm⁻¹ bond corresponds to the Si-OH elongation band (A. Ensafi et al,

Electroanalysis, 2017, Vol 29, pp. 2461-2469; S.K. Parida et al, Advances in Colloid and Interface Science, 2006, Vol 121, pp. 77-10.).

UV- visible spectroscopy: In the UV-Visible spectrum (Figure 21), the loss of absorbance in the visible wavelengths proves that the nanoparticles of bismuth are coated with a layer of silica (the bismuth nanoparticles are more black).

Conclusion: Silanization by tetraethoxysilane of Bi NPs is efficient. The presence of silica on the surface of Bi NPs is demonstrated by above characterizations.

Example 18: Silanization by 2-[3-(triethoxysilyl)propyl]succinic acid (TEPSA)

Purpose: Coat Bi NPs with 2-[3-(triethoxysilyl)propyl]succinic acid.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. A first silanization is performed with tetraethoxysilane (TEOS) : a solution of tetraethoxysilane (48 mM, 62 mg, 0.3 mmol, 40 eq) diluted in ethanol (6 ml) is added dropwise to a aqueous solution of bismuth nanoparticles (1.2 mM, 6 ml, 0.007 mmol, 1 eq) at pH 10 diluted in absolute ethanol (15 ml). After 24 hours of strong agitation, Bi@SiO₂ nanoparticles are isolated and washed with absolute ethanol by ultrafiltration. The treatment is terminated when the filtrate reaches a conductivity of 0.4 μS. A second silanization is performed with 2-[3-(triethoxysilyl)propyl]succinic acid (TEPSA): a solution of bismuth nanoparticles (5 ml, 1 mM, 0.015 mmol, 1 eq) at pH 7 is diluted in DMF (10 ml) and this solution is then concentrated by removing water. Then, an aqueous solution of NaOH (50 μl, 1M) and TEPSA (0.060 mmol, 4 eq) are added. The mixture is heated at 100 °C for 24h. The bismuth nanoparticles are characterized by a measurement of DLS and of Zeta potential. The solution is then ultrafiltered with a 30 kDa filter and an aqueous solution at pH 10. After the treatment, the bismuth nanoparticles are characterized by DLS and zeta potential (Table 17).

Table 17: Size and zeta potential of 2-[3-(triethoxysilyl)propyl]succinic acid coated metallic bismuth nanoparticles synthesized by silanization.

	bismuth nanoparticles citrate before silanization	bismuth nanoparticles after silanization, in water	bismuth nanoparticles after ultrafiltration, in water
Size (nm) and population distribution (percentage) - DLS	42 ± 20 (100%)	124 ± 72 (100%)	56 ± 30 (100%)
Zeta potential	-47 mV	-29 mV	-30 mV

Conclusion: Silanization by 2-[3-(triethoxysilyl)propyl]succinic acid (TEPSA) of bismuth nanoparticle is efficient.

5 **Example 19: Silanization by 3-aminopropyltriethoxysilane (APTES)**

Purpose: Coat bismuth metallic nanoparticles with 3-aminopropyltriethoxysilane.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. A first silanization is performed with tetraethoxysilane: a solution of tetraethoxysilane (48 mM, 62 mg, 0.3 mmol, 40 eq.) diluted in ethanol (6 ml) is added dropwise to a aqueous solution of bismuth nanoparticles (1.2 mM, 6 ml, 0.007 mmol, 1 eq.) at pH 10 diluted in absolute ethanol (15 ml). After 24 hours of strong agitation, Bi@SiO₂ nanoparticles are isolated and washed with absolute ethanol by ultrafiltration. The treatment is terminated when the filtrate reaches a conductivity of 0.4 µS. A second silanization is then performed with 3-aminopropyltriethoxysilane: a solution of 3-aminopropyltriethoxysilane (APTES) (1.5 µl, 0.07 mmol, 0.5 eq.) diluted in ethanol (6 ml) then this solution is added dropwise to the solution containing Bi@SiO₂ in ethanol (20 ml). After 24 hours of strong agitation, the bismuth nanoparticles are precipitated by ethanol.

DLS and zeta potential measurements (Table 18).

Table 18: Size and zeta potential of 3-aminopropyltriethoxysilane coated metallic Bi NPs.

Batch	Size (nm) and population distribution (percentage) measure by DLS	Zeta potential (mV)
1	133± 30 (100%)	27 mV

Infrared spectroscopy: In the infrared spectrum (Figure 22), the band of 3630 cm^{-1} corresponds to the N-H bond as well as to the band of 1570 cm^{-1} . The bands 2900 and 2950 cm^{-1} correspond to the vibration mode of the $\text{C}_{\text{sp}^3}\text{-H}$ bond. The large band around 1000 cm^{-1} corresponds to Si-O-Si and C-N vibration modes.

UV-visible spectroscopy: In the UV-visible spectrum (Figure 23), the loss of absorbance in the visible wavelengths proves that the nanoparticles of bismuth are coated with a layer of silica.

Conclusion: Silanization by 3-aminopropyltriethoxysilane of Bi NPs is efficient. The presence of the coating is demonstrated by the analysis performed.

Exemple 20: Silanization by (3-trimethoxysilyl)propyl methacrylate

Purpose: Coat bismuth metallic nanoparticles with (3-trimethoxysilyl) propyl methacrylate.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. A first silanization is performed with tetraethoxysilane: a solution of tetraethoxysilane (48 mM, 62 mg, 0.3 mmol, 40 eq.) diluted in ethanol (6 ml) is added dropwise to a solution of bismuth nanoparticles (1.2 mM, 6 ml, 0.007 mmol, 1 eq.) at pH 10 diluted in absolute ethanol (15 ml). After 24 hours of strong agitation Bi@SiO₂ nanoparticles are isolated and washed with absolute ethanol by ultrafiltration. The treatment is terminated when the filtrate reaches a conductivity of 0.4 μS . A second silanization is then performed with 3-(propyltrimethoxysilane)methacrylate: a solution of 3-(propyltrimethoxysilane)methacrylate (5 eq.) diluted in ethanol (6 ml) the this solution is added dropwise to a solution containing the Bi@SiO₂ nanoparticles diluted in ethanol (20

ml). After 24 hours of strong agitation, the bismuth nanoparticles are ultrafiltered with ethanol.

DLS and zeta potential measurements (Table 19).

Table 19: Size (DLS) and zeta potential of 3-(propyltrimethoxysilane) methacrylate coated bismuth metallic nanoparticles.

Size (nm) and population distribution (percentage) (ethanol) - DLS	potential zeta (ethanol)
48±17 (100%)	-25 mV

Infrared spectroscopy: In the IR spectrum (Figure 24), the bands 2920 and 2854 cm⁻¹ are characteristic of the vibration modes of the sp³ carbons CH₃ and CH₂ respectively. The 1050 cm⁻¹ band is characteristic of the Si-O vibration mode.

Conclusion: Silanization by 3-(propyltrimethoxysilane)methacrylate of Bi NPs is efficient. The presence of the coating is demonstrated by the analysis performed.

Example 21: Silanization by 2,2,1,1-tridecafluoro-n-octylsilane

Purpose: Coat bismuth metallic nanoparticles with 2,2,1,1-tridecafluoro-n-octylsilane.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. A first silanization is performed with tetraethoxysilane: a solution of tetraethoxysilane (48 mM, 62 mg, 0.3 mmol, 40 eq.) diluted in ethanol (6 ml) is added dropwise to a solution of bismuth nanoparticles (1.2 mM, 6 ml, 0.007 mmol, 1 eq.) at pH 10 diluted in absolute ethanol (15 ml). After 24 hours of strong agitation Bi@SiO₂ nanoparticles are isolated and washed with absolute ethanol by ultrafiltration. The treatment is terminated when the filtrate reaches a conductivity of 0.4 µS. A second silanization is then performed with 2,2,1,1-tridécafluoro-n-octylsilane: a solution of 2,2,1,1-tridecafluoro-n-octylsilane (0.7 mmol, 5 eq.) diluted in ethanol (6 ml) is added dropwise to the solution containing Bi@SiO₂ in ethanol (20 ml). After 24 hours of strong agitation, the bismuth nanoparticles are precipitated by ethanol.

DLS, zeta potential and stability measurements (Table 20).

Table 20: Size (DLS), zeta potential and stability of 2,2,1,1-tridecafluoro-n-octylsilane coated bismuth metallic nanoparticles.

DLS size (nm) and population distribution (percentage)	Zeta potential (mV)	Solubility in PFOB
63±23 (100%)	-25 mV	yes
54 ±19 (100%)	-19 mV	yes

Infrared spectroscopy: In the IR spectrum the bond around 1156 cm⁻¹ corresponds to CF₂ and the large bond around 1074 cm⁻¹ corresponds to Si-O-Si.

- 5 Conclusion: Silanization by 2,2,1,1-tridecafluoro-n-octylsilane of Bi NPs is efficient. The presence of the coating is demonstrated by the analysis performed.

Example 22: Sterilization of Bi NPs by moist heat at 121°C during 15 minutes

Purpose: Sterilize bismuth metallic nanoparticles and assess stability under such treatment.

- 10 Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The sterilization test was then carried out as follows: a solution comprising bismuth nanoparticles (4 mM), a citrate buffer (2 mM) at pH 7 and a manitol in solution (300 mM) is prepared. Samples (2 ml) are taken and put into ampoules to be sterilized by autoclaving. The sterilization is performed at 121°C for 15 minutes. Measures of DLS, pH, Zeta potential and osmolality were performed before and after sterilization.

Table 21: Size (by DLS), zeta potential, pH and osmolality of citrate-coated Bi NPs before and after sterilization.

	Size (nm) and population distribution (percentage) – DLS	zeta potential (mV)	pH	Osmolality (mOsm/kg)
Before sterilization	28±10 (100%)	-39	7.10	278
After sterilization	16±4 (100%)	-28	7.01	255

- 20 Conclusion: The Bi NPs of the invention can be sterilized by moist heat at 121°C for 15 minutes without degradation.

Example 23: Degradation of Bi NPs in power

Purpose: Assess the stability of bismuth metallic nanoparticles in powder.

Protocol: The nanoparticles are synthesized in continuous flow with thermal activation with the valve 3 (conicity 3°) as described in Example 8. The degradation test of nanoparticles in powder form was performed as follows: the nanoparticles were lyophilized and then stored in powder form for 1 month at room temperature. Measures of nanoparticle size by DLS were performed before and after lyophilization and after storage under powder form.

Table 22: Size (DLS) of citrate coated Bi NPs before lyophilization, after lyophilization and after storage 1 month under powder form.

	Before lyophilisation	After lyophilisation	Stored 1 month at powder
Size (nm) and population distribution (percentage) - DLS	54 ±26 (100%)	41 ± 18 (100%)	44 ± 17 (100%)

Conclusion: The Bi NPs of the invention can be stored in powder for at least 1 month.

Example 24: Oil-in-water emulsion comprising nanoparticles

Purpose: Providing an oil-in-water emulsion comprising nanoparticles incorporated in the oily phase.

Protocol: In a bottle, glycerol (0.123 ml) and poloxamer P188 (166 mg) were added to water (4.8 ml), the solution is heated to 50 °C. In another bottle, lipophilic bismuth metallic nanoparticles of example 15b (6 mg) were solubilized in miglyol (1.075 ml) at room temperature. The aqueous solution was added to the oil. This mixture was homogenized using ultra-turrax at 20,500 rpm.

Conclusion: The emulsion is stable during more than 14 days

CLAIMS

1. A process for manufacturing biocompatible bismuth (0) nanoparticles comprising a core comprising bismuth (0), surrounded by at least one coating layer adsorbed thereon, said process being characterized in that it comprises the steps of:

- 5 a) preparing an aqueous solution with a pH greater than 12, said aqueous solution comprising:

- a water-soluble bismuth precursor of formula Bi(III)-L totally dissolved in the solution, wherein L is a stabilizing group selected from polycarboxylic acid and thiocarboxylic acid; preferably the water-soluble bismuth precursor is selected from bismuth (III) citrate, bismuth (III) lipoate and bismuth (III) dimercaptosuccinate; more preferably the water-soluble bismuth precursor is bismuth (III) citrate;
- a water-soluble reducing agent selected from monosaccharides;
- optionally one or more coating agent of formula X-L'; and
- 15 - water;

wherein:

X is a cation, preferably a pharmaceutically acceptable cation, more preferably a cation selected from hydrogen cation, sodium and ammonium; and

L' is selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, and biotargeting derivatives thereof of formula L¹-A-L², wherein:

L¹ is selected from polycarboxylic acid, thiocarboxylic acid and thioalcohol,

L² is a biotargeting group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol and zwitterionic groups; and
- 25 - a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances; and

A is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;

- b) heating the aqueous solution obtained in step a), and
 - c) recovering the bismuth (0) nanoparticles from the aqueous solution obtained in step b).
2. The process according to claim 1, wherein the polycarboxylic acid is citric acid; and the thiocarboxylic acid is dimercaptosuccinic acid, glutathione or lipoic acid.
 3. The process according to claim 1 or claim 2, wherein the monosaccharide water-soluble reducing agent is glucose.
 4. The process according to any one of claims 1 to 3, wherein the process further comprises a step of sterilization, preferably a step of sterilization under heating.
 5. The process according to any one of claims 1 to 4, wherein the recovering of step c) is made by filtration, preferably by ultrafiltration.
 6. The process according to any one of claims 1 to 5, wherein said process comprises an additional step of coating-exchange during which the bismuth (0) nanoparticles

of step c) are put in presence of a different coating agent of formula $X''-L''$, wherein:

X'' is a cation, preferably a pharmaceutically acceptable cation, more preferably a cation selected from hydrogen cation, sodium and ammonium; and

5 L'' is a stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1''}-A''-L^{2''}$, wherein:

10 $L^{1''}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioalcohol, thioamine, polyamine, aminosaccharide, diphosphonopentanoic acid, polyphosphoric acid and cyclodextrine, and

$L^{2''}$ is a group selected from:

- 15 - a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
- a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or
- 20 polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and
- 25 - a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, selected from saturated or unsaturated C_3F_7 to $C_{18}F_{37}$ perfluorinated chains and partially fluorinated alkyl chains; and

30 A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of

-O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-.

7. The process according to any one of claims 1 to 6, wherein said process comprises an additional step of silanization; optionally followed by a step of functionalization of silica by one or more organohydroxysilane group functionalized by:
- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
 - a bioactive group, selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
 - a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from hydroxysilane groups such as alkyl hydroxysilane, metacrylate hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octohydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane, octodecylhydroxysilane or methacryloxypropylhydroxysilane; and

- a fluorophilic group capable of solubilizing Bi NPs in an fluorous phase, selected from perfluorinated organohydroxysilane groups and partially fluorinated alkylhydroxysilane groups such as fluoromethylhydroxysilane 2-fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxysilane, 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-pentafluorobutylhydroxysilane.
8. The process according to any one of claims 1 to 7, wherein said process is carried out in continuous flow.
9. Bismuth (0) nanoparticles obtained by the process according to any one of claims 1 to 8.
10. A bismuth (0) nanoparticle comprising:
- a core comprising bismuth (0),
 - surrounded by at least one coating layer adsorbed on the core, comprising:
 - (1) at least one stabilizing group selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid, cyclodextrine and derivatives thereof of formula $L^{1'}-A''-L^{2'}$, wherein:
 - $L^{1'}$ is selected from polycarboxylic acid, thiocarboxylic acid, thioamine, polyamine, aminosaccharide, polyphosphoric acid and cyclodextrine, and
 - $L^{2''}$ is a group selected from:
 - a hydrophilic group having an effect on biodistribution or on macrophage capture selected from aminoalcohol, polyethylene glycol or zwitterionic groups;
 - a bioactive group selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or

minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;

- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from saturated or unsaturated C_6H_{13} to $C_{18}H_{37}$ fatty alkyl chains such as dodecyl, tetradecyl, hexyldecyl or octadecyl; and
- a fluorophilic group capable of solubilizing Bi NPs in a fluorous phase, selected from saturated or unsaturated C_3F_7 to $C_{18}F_{37}$ perfluorinated chains and partially fluorinated alkyl chains; and

A'' is a linking group selected from single bond; -O-; -S-; -NH-; -(CO)O-; -NHCO-; -CONH-; -NHCONH-; -NHCSNH-; -SO₂NH-; -NHSO₂-; squarate; C1-C6 alkylene; polyethylene glycol; and -P¹-I-P²-, wherein P¹ and P² are independently selected from the group consisting of -O-, -S-, -NH-, a single bond, -(CO)O-, -NHCO-, -CONH-, -NHCONH-, -NHCSNH-, -SO₂NH-, -NHSO₂-, and squarate; and wherein I is selected from the group consisting of: alkylene; alkoxyalkylene; polyalkoxyalkylene; alkylene interrupted with one or several squarates; alkylene interrupted with one or several aryls; alkylene interrupted with one or several heteroaryls; alkylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; alkenylene; alkynylene; alkenylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-; and alkynylene interrupted with one or several groups selected from -NH-, -O-, -CO-, -NH(CO)-, -(CO)NH-, -O-(CO)-, and -(OC)O-;

and/or

(2) at least one organohydroxysilane group functionalized by a group selected from:

- a hydrophilic group having an effect on biodistribution or on macrophage capture, selected from aminoalcohol, polyethylene glycol or zwitterionic groups;

- a bioactive group, selected from antibody, such as polyclonal or monoclonal antibody, hybrid or chimeric antibody, single-domain antibody, dimeric or trimeric antibody fragment construct or minibody; peptide; pseudopeptide; protein; nucleic acid; mono or polysaccharide; and pharmacologically active substances;
- a hydrophobic group capable of solubilizing Bi NPs in an oily phase, selected from hydroxysilane groups such as alkyl hydroxysilane, metacrylate hydroxysilane or ureido hydroxysilane; vinyl hydroxysilane groups such as ethylhydroxysilane, propylhydroxysilane, butylhydroxysilane, pentylhydroxysilane, hexylhydroxysilane, heptylhydroxysilane, octylhydroxysilane, nonylhydroxysilane, decylhydroxysilane, dodecylhydroxysilane, tetradecylhydroxysilane, hexyldecylhydroxysilane, octodecylhydroxysilane or methacryloxypropylsilane; and
- a fluorophilic group capable of solubilizing Bi NPs in a fluoruous phase, selected from perfluorinated organohydroxysilane groups or partially fluorinated alkylhydroxysilane groups such as fluoromethylhydroxysilane 2-fluoroethylhydroxysilane, 2,2-difluoroethylhydroxysilane, 2,2,2-trifluoroethylhydroxysilane or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluorooctylhydroxysilane, 3,3,4,4,5,5,6,6,6-nonafluorohexylhydroxysilane, 4,4,5,5,6,6,7,7,7-nonafluoroheptylhydroxysilane, 2,2,3,3,3-pentafluoropropylhydroxysilane, 3,3,4,4,5,5,5-heptafluoropentylhydroxysilane, 3,3,4,4,4-pentafluorobutylhydroxysilane;

and

the nanoparticle comprises from 1 to 40 stabilizing groups per nm²,

the nanoparticle size is smaller than 40 nm measured by Transmission Electron Microscopy (TEM);

the ratio of the nanoparticle hydrodynamic diameter measured by dynamic light scattering (DLS) in number to the nanoparticle size measured by TEM is ranging from 1 to 45, and

the nanoparticle does not contain boron derivatives.

11. A composition comprising bismuth (0) nanoparticles as defined in claim **10** and a carrier.
12. Delivery device comprising a container and an injection device, the said container containing a composition as defined in claim **11**.
13. Bismuth (0) nanoparticles according to claim **10** for use as medicament; preferably as radiosensitizer agent in radiotherapy or in photothermal therapy, or as biocide agent.
14. Non-therapeutic use of bismuth (0) nanoparticles according to claim **10** as biocide agent; preferably as bactericide, antimicrobial, antibacterial, antimycotical, fungicide, antiparasitic, antibiofilm, antibiofouling or antiviral agent.
15. Use of bismuth (0) nanoparticles according to claim **10** as radioopacifying agent, preferably as X-ray contrast agent for medical imaging or as radioopacifying agent in medical devices.

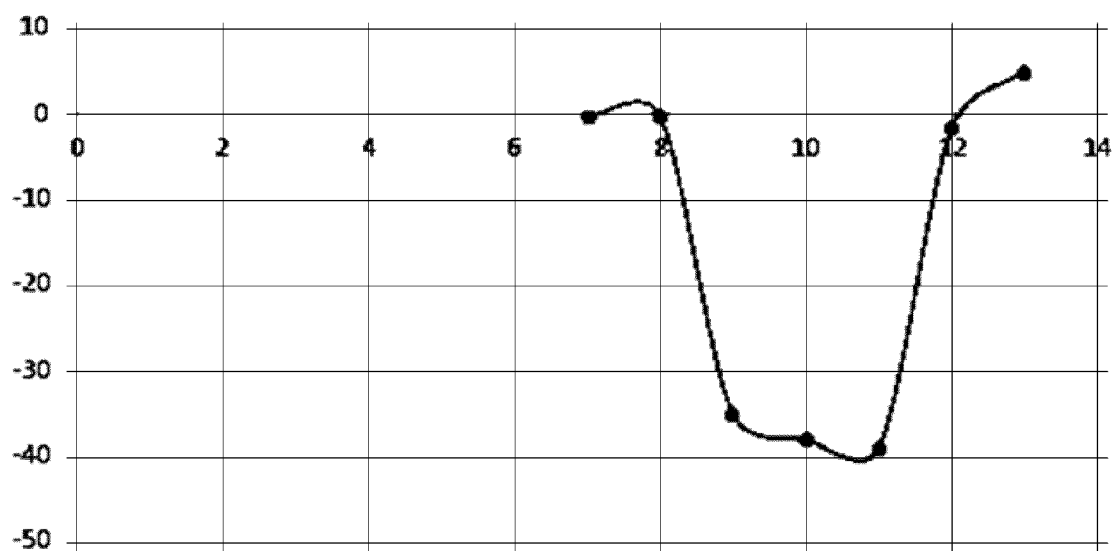


FIG. 1

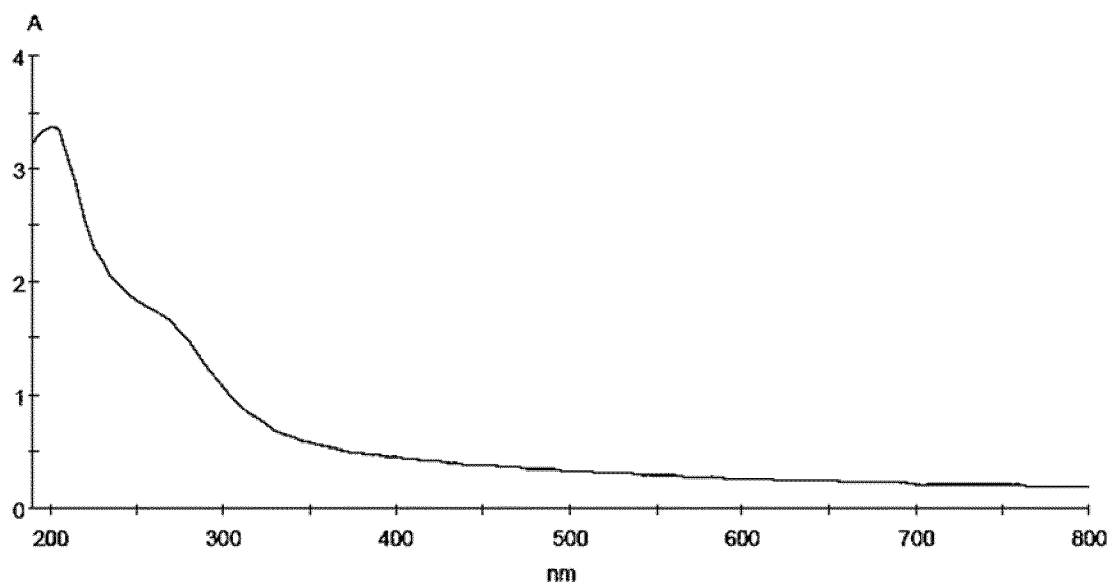


FIG. 2

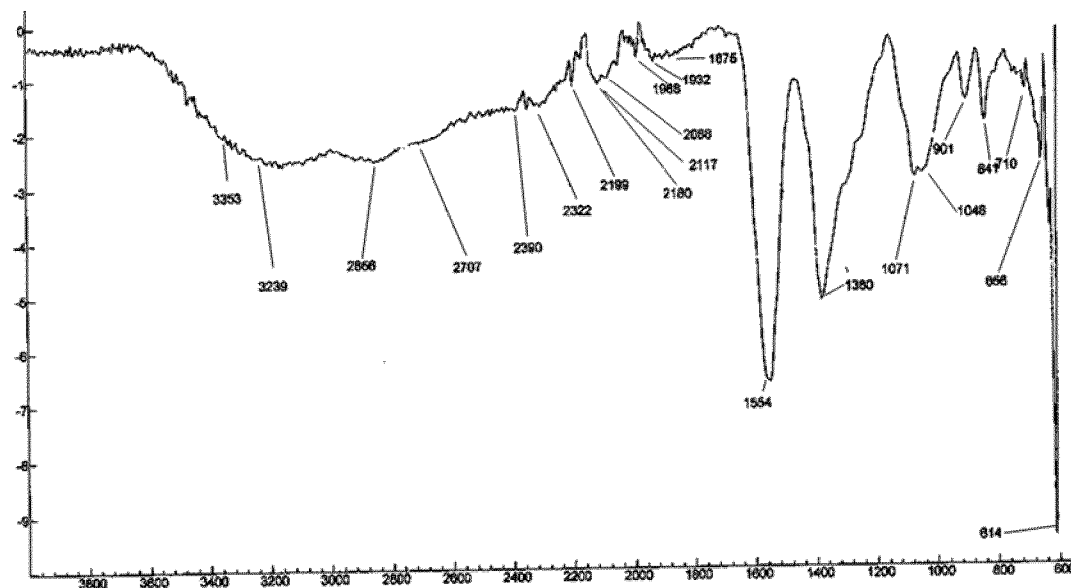


FIG. 3

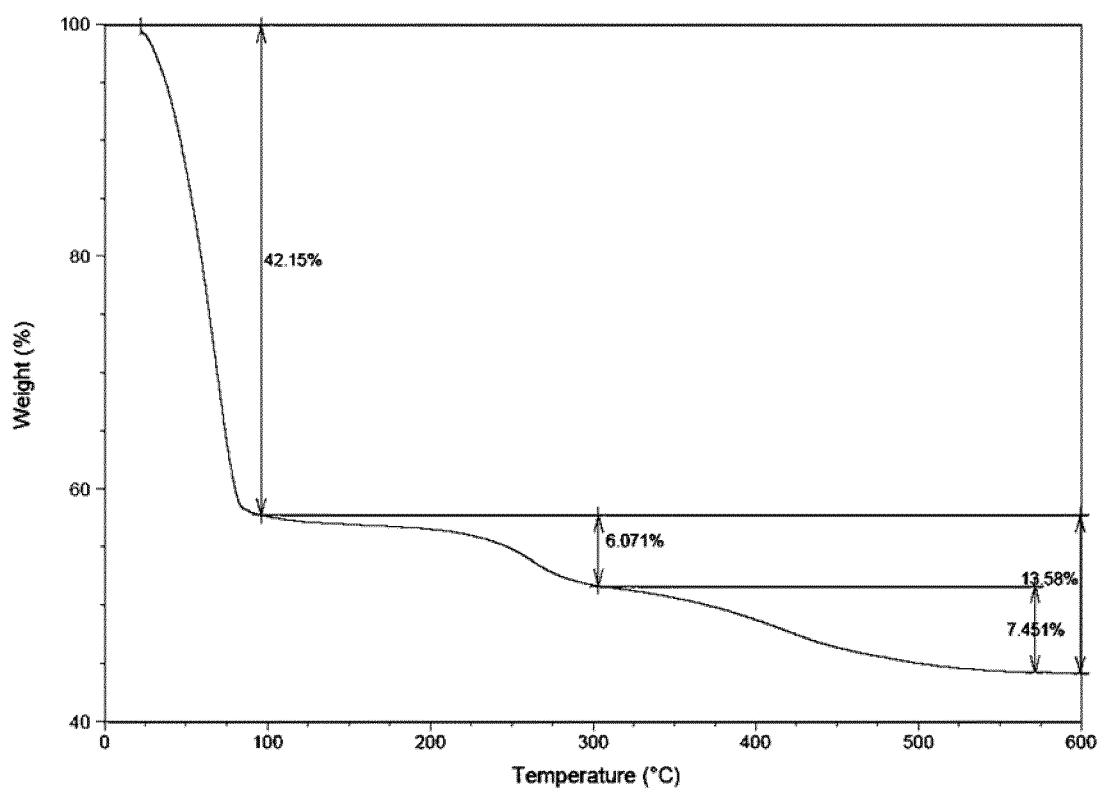


FIG. 4

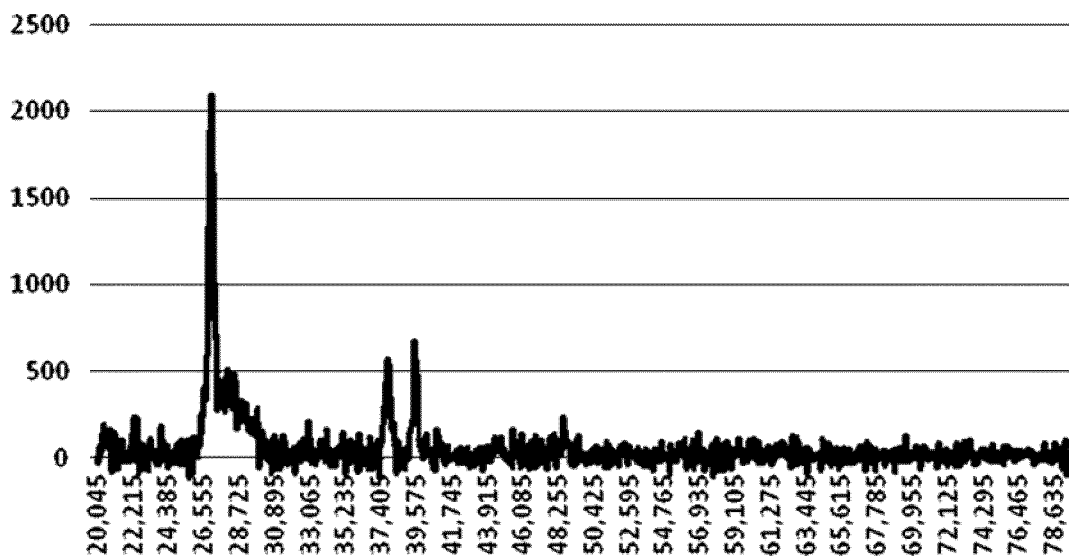


FIG. 5

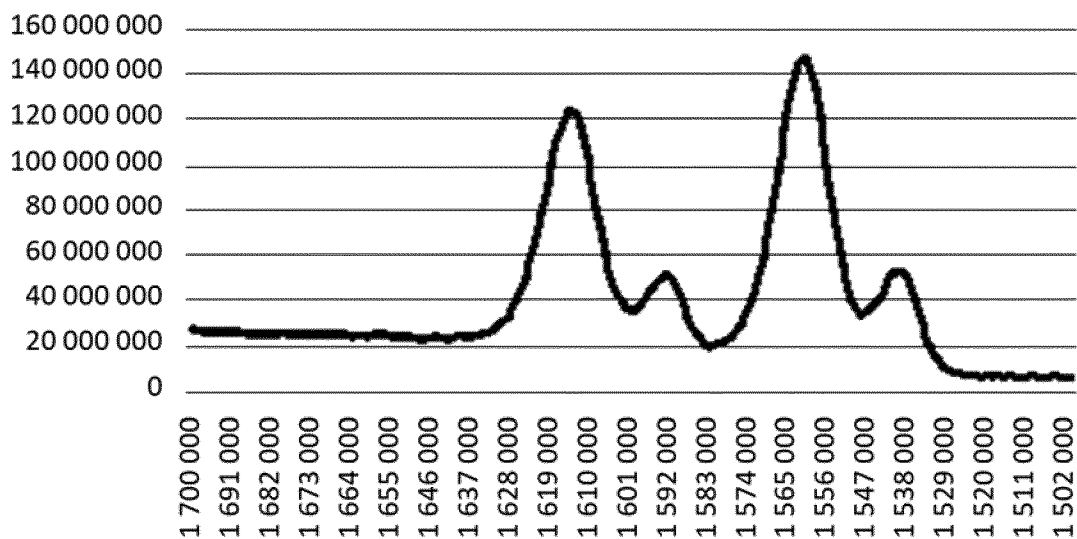


FIG. 6

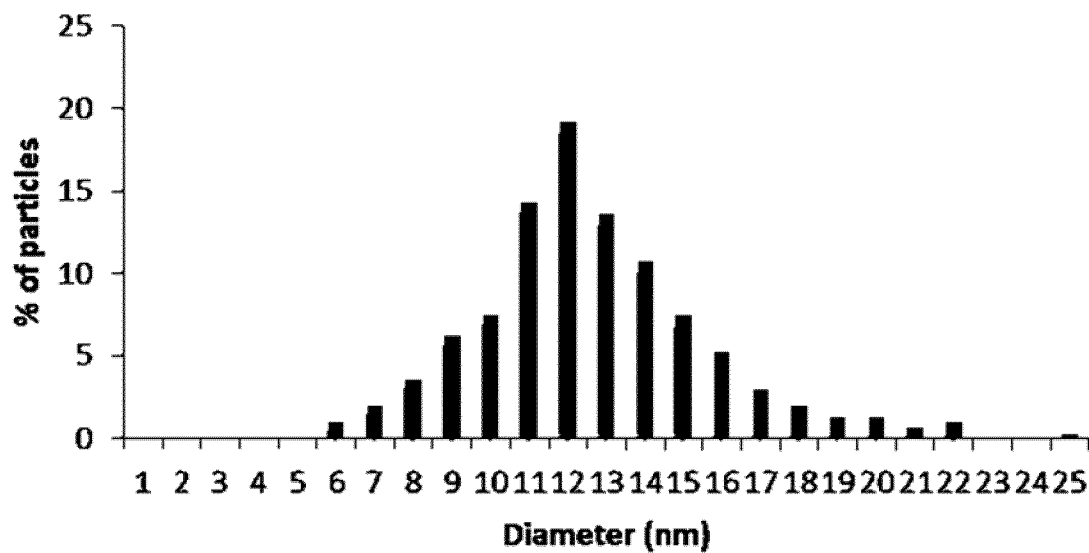


FIG. 7

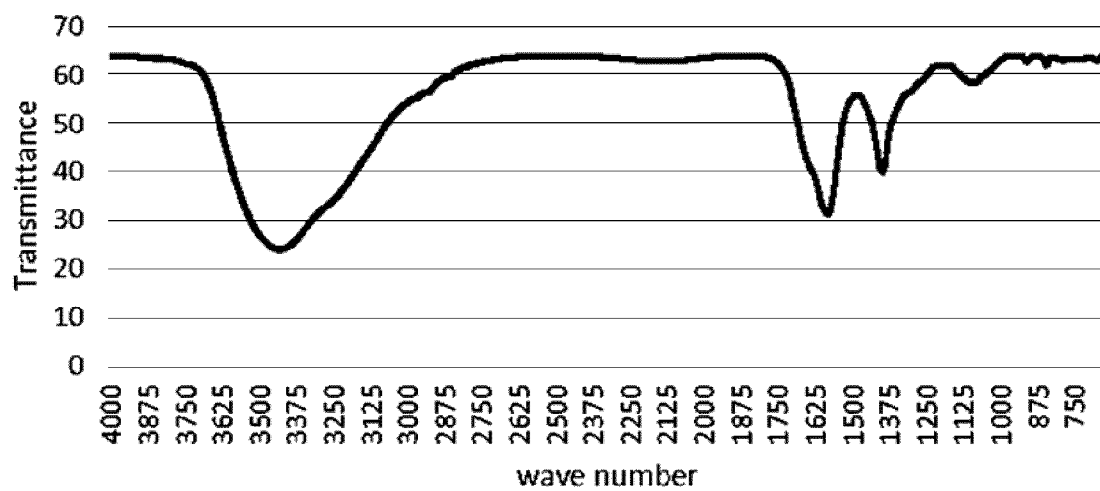


FIG. 8

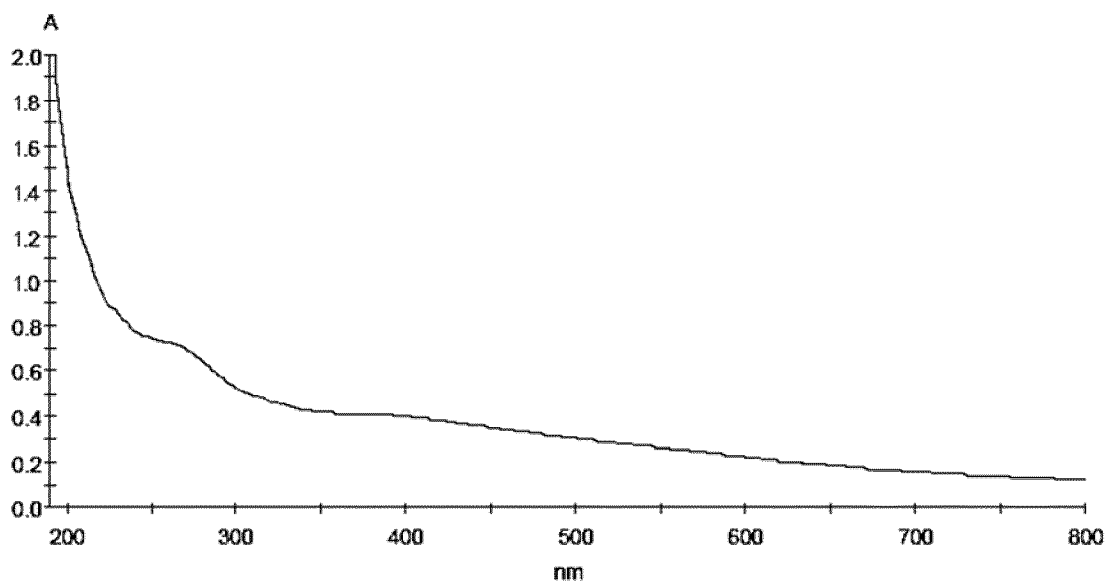


FIG. 9

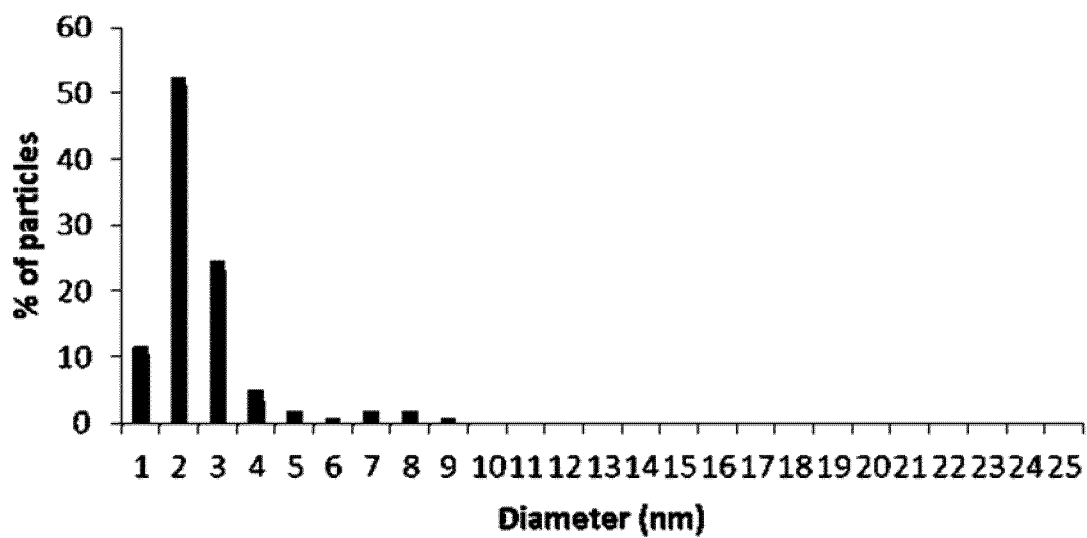


FIG. 10-1

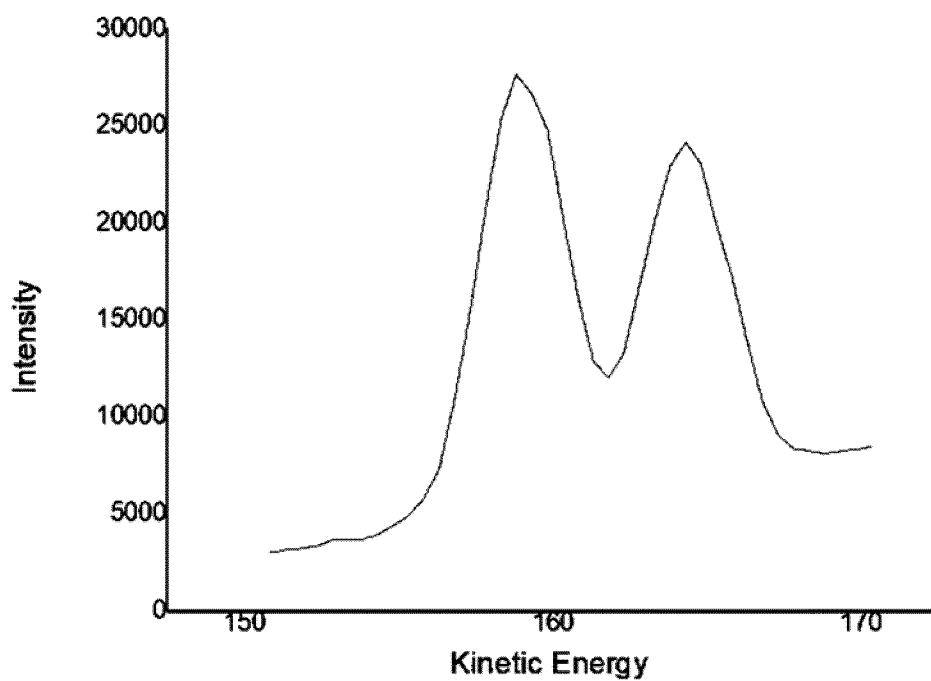


FIG. 10-2

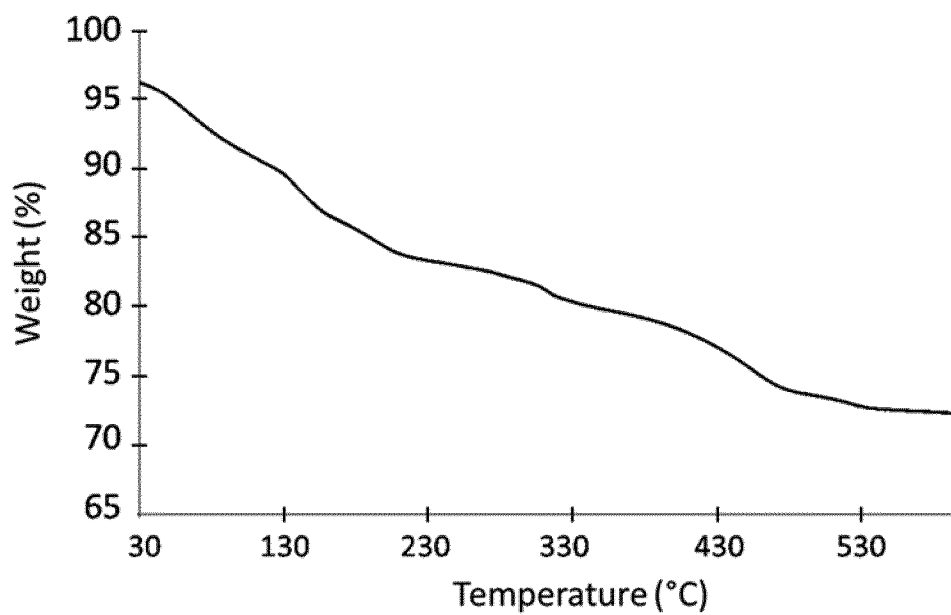


FIG. 10-3

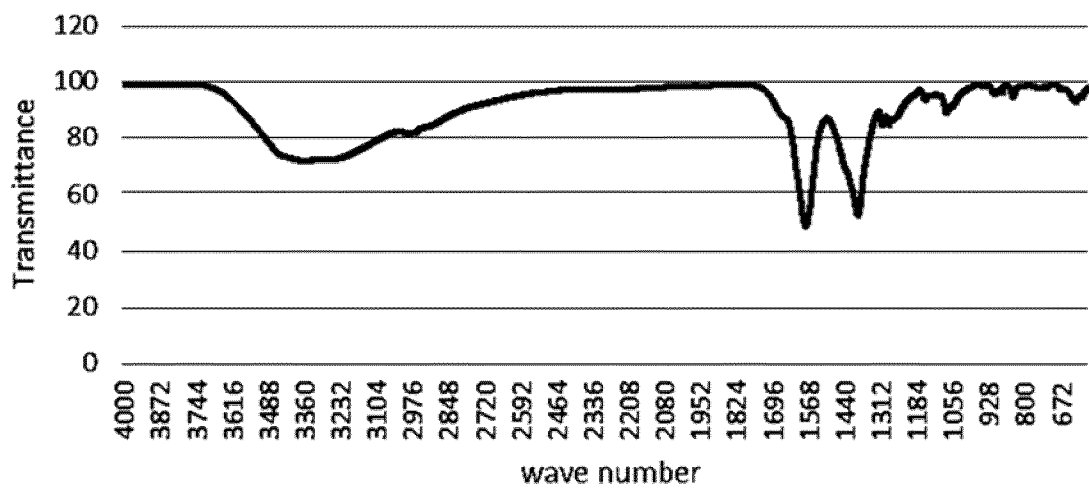


FIG. 11

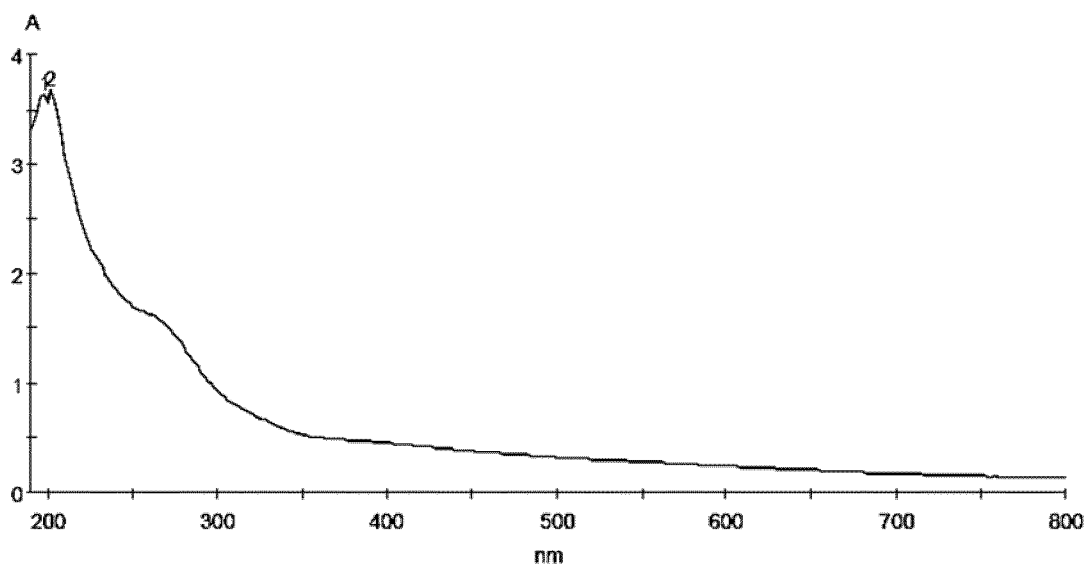


FIG. 12-1

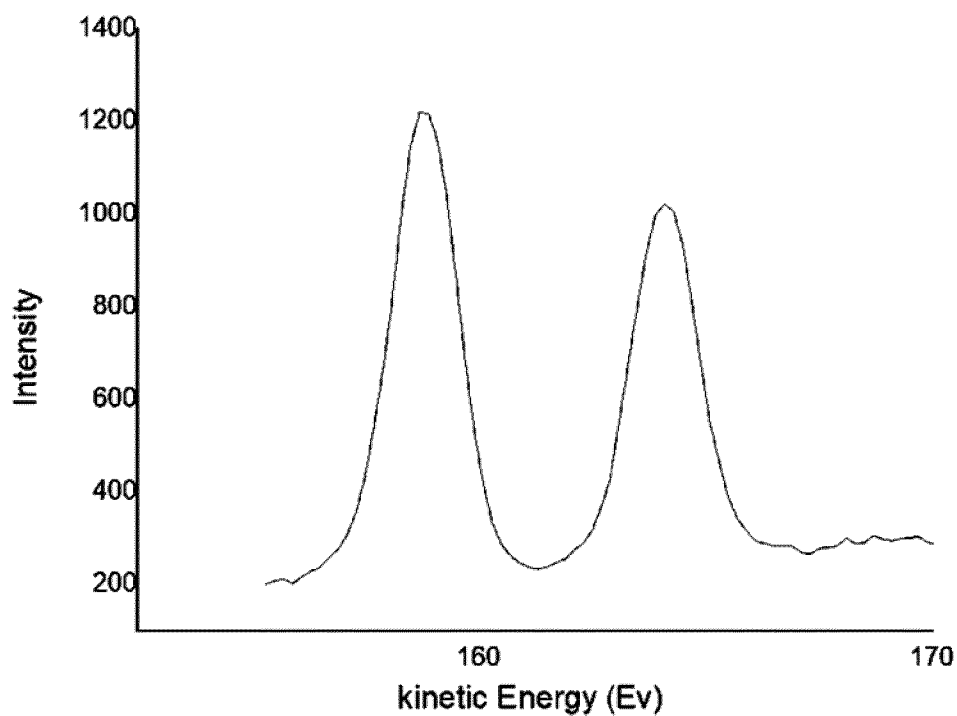


FIG. 12-2

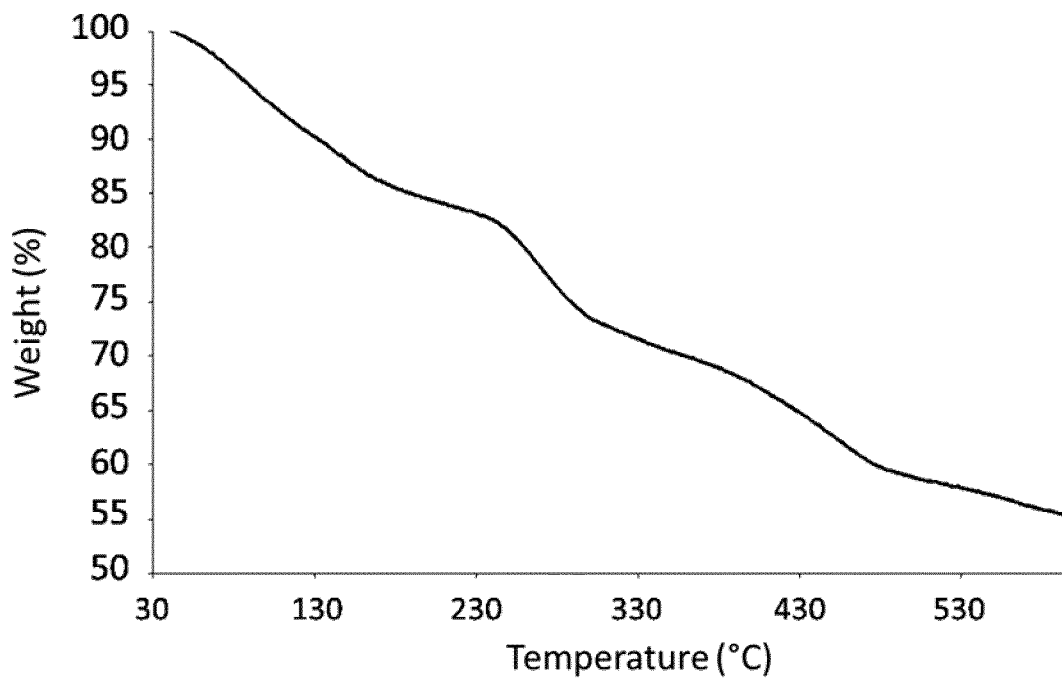


FIG. 12-3

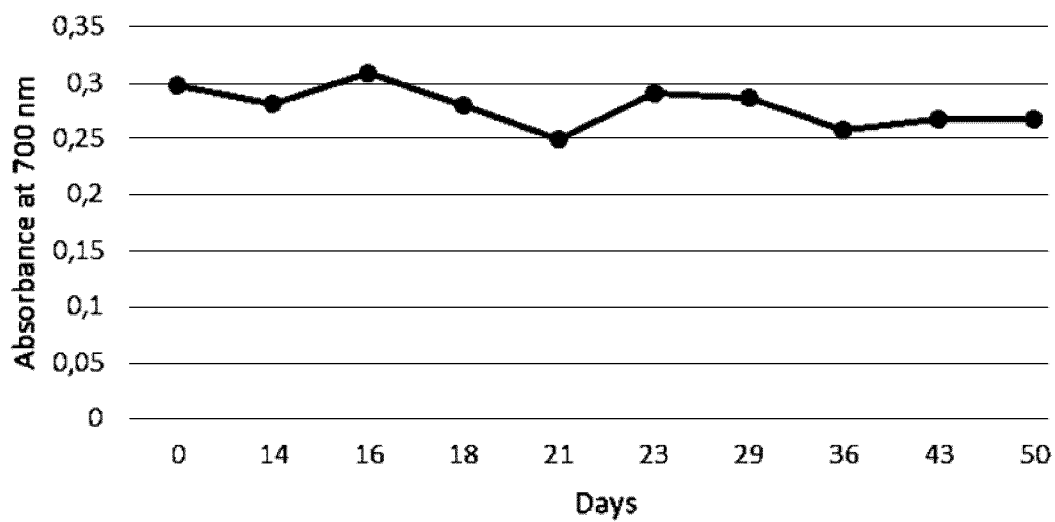


FIG. 13

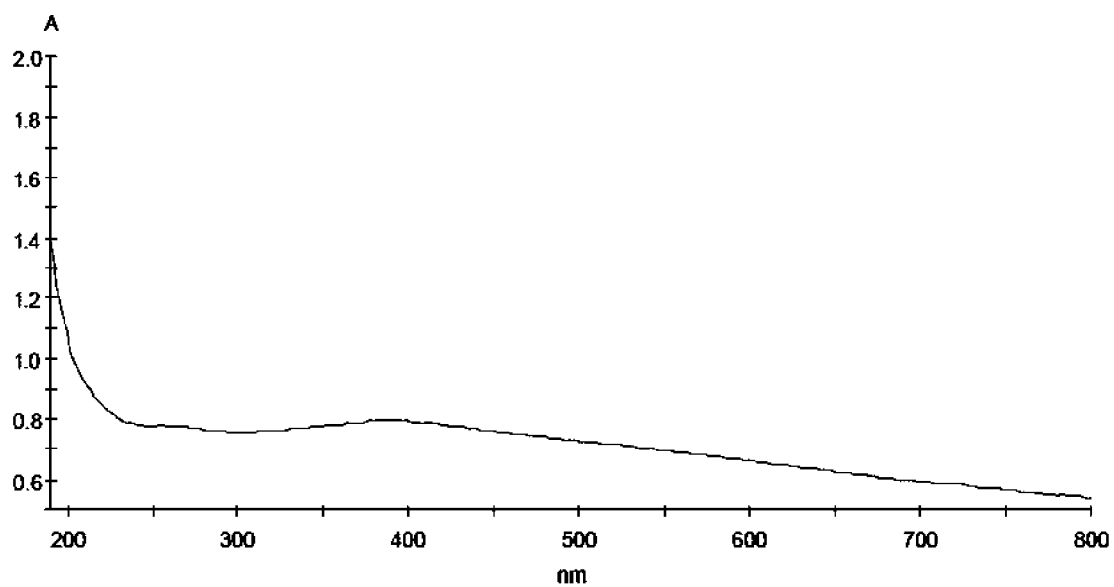


FIG. 14

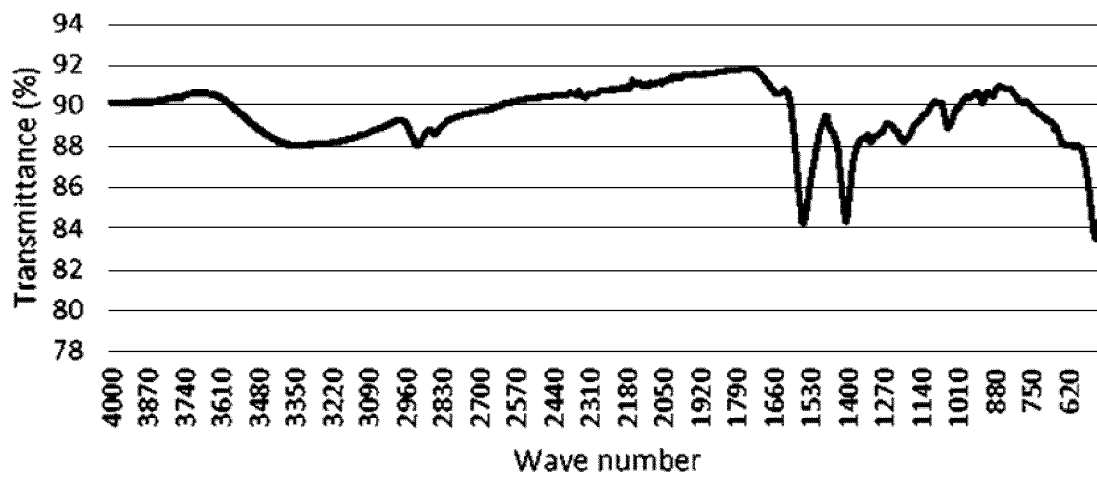


FIG. 15

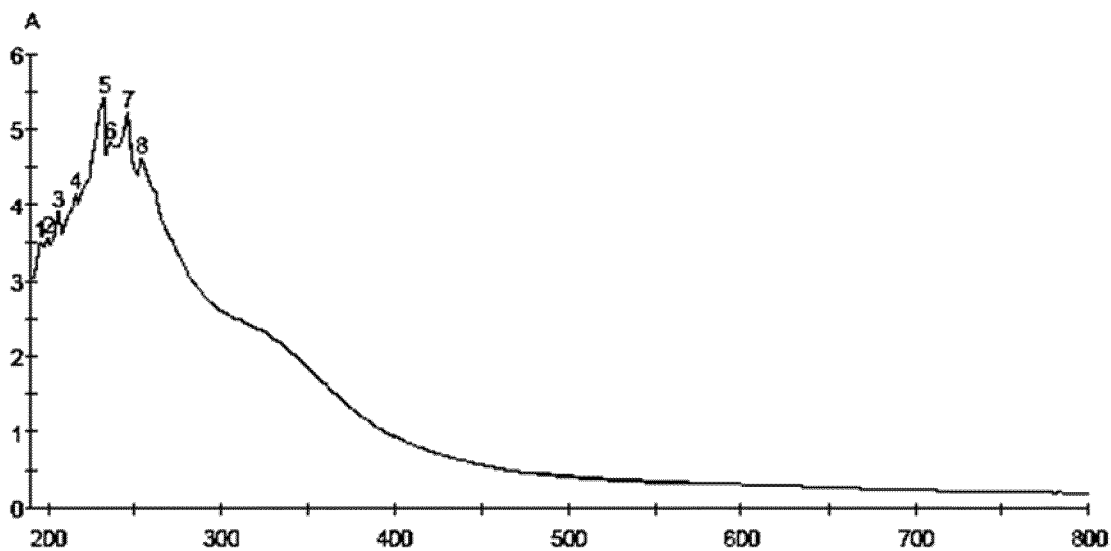


FIG. 16

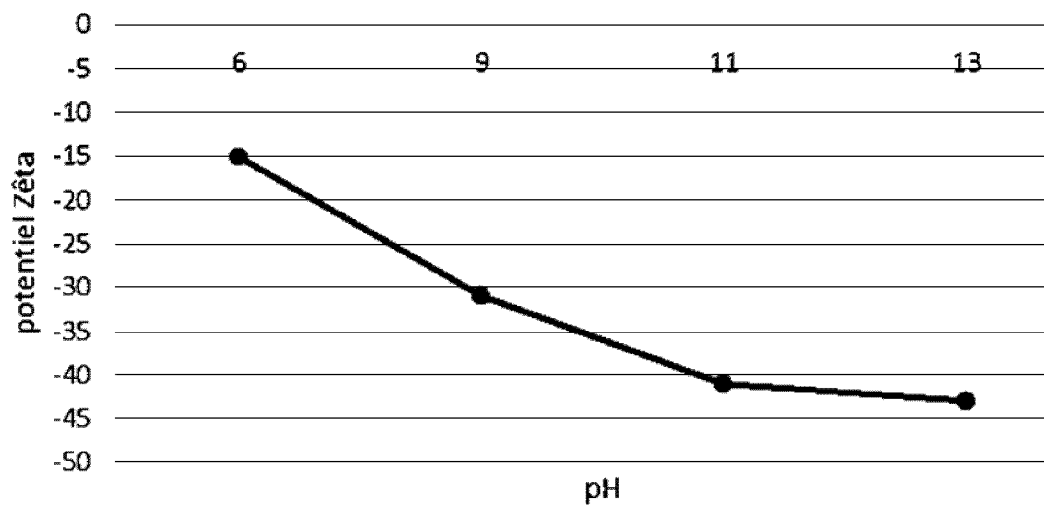


FIG. 17

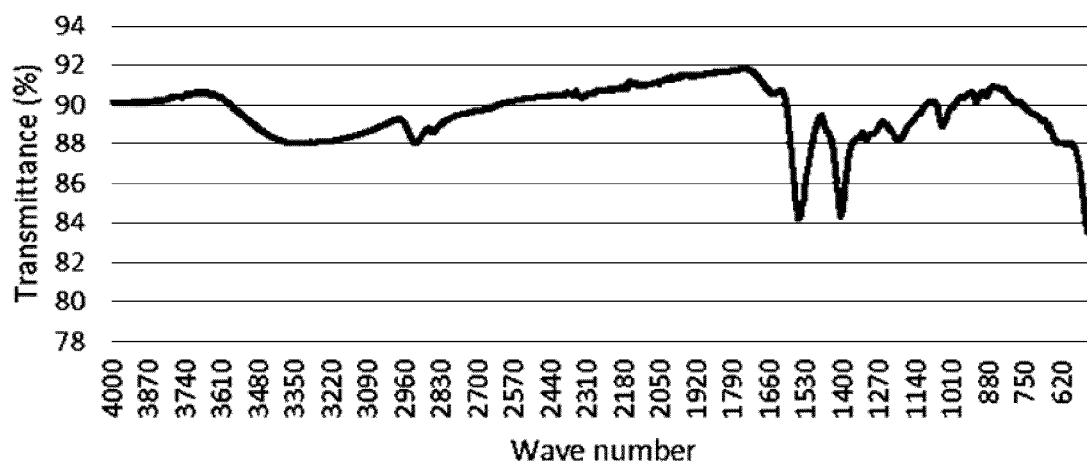


FIG. 18

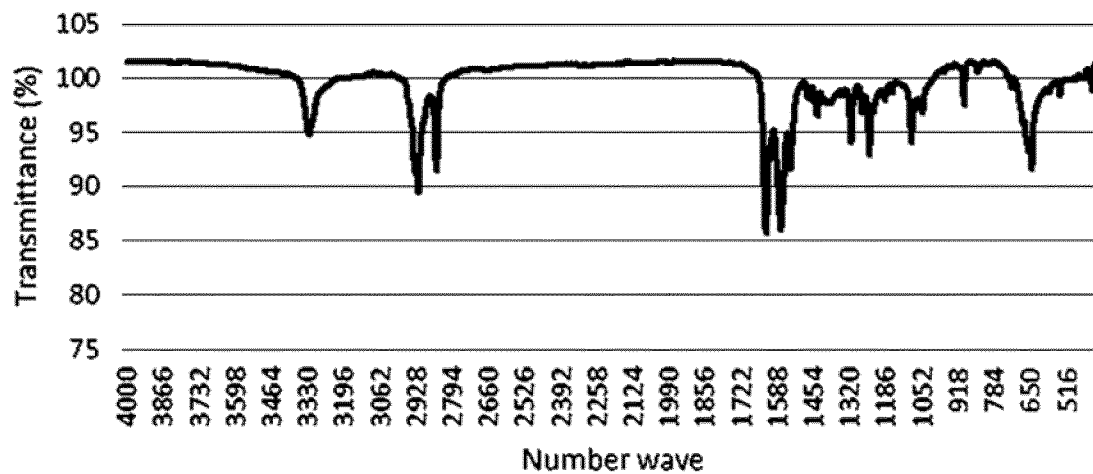


FIG. 19-1

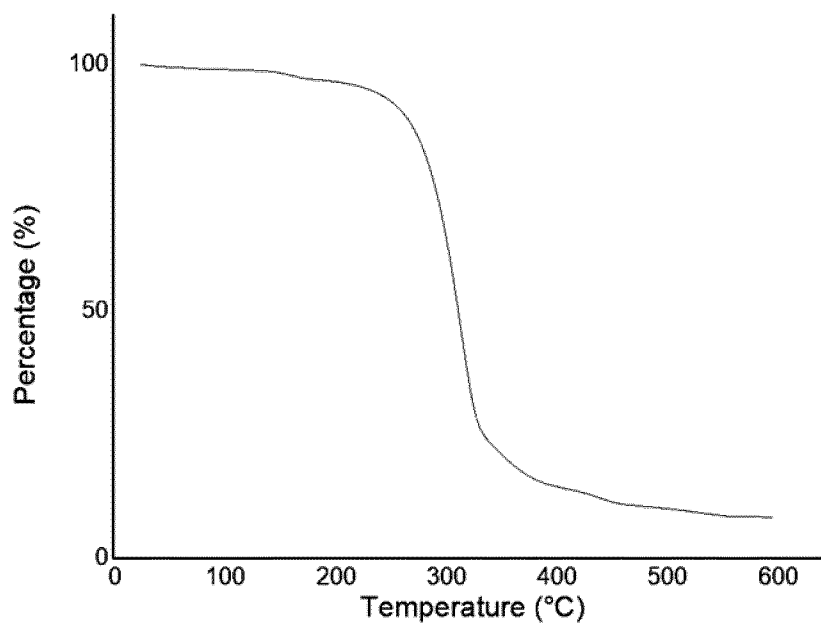


FIG. 19-2

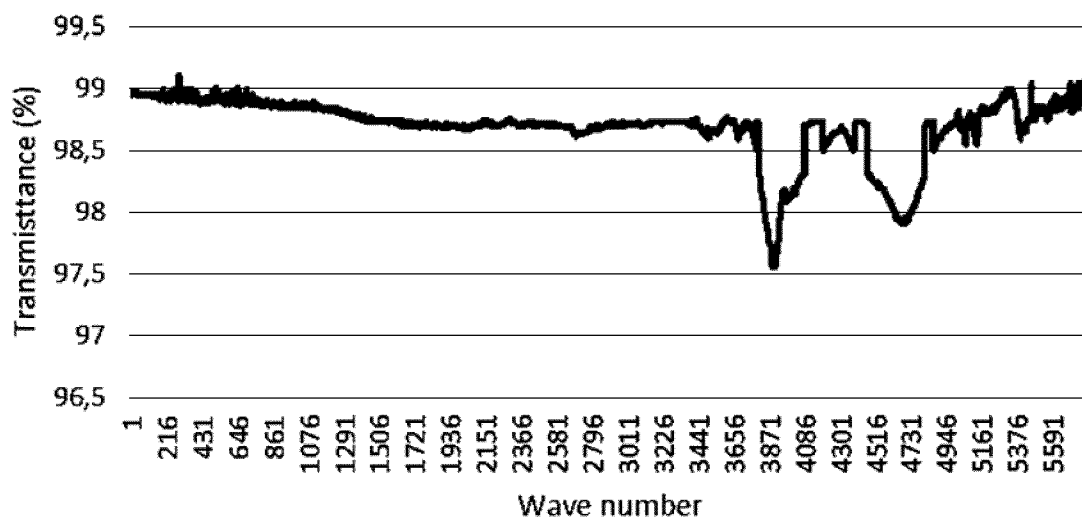


FIG. 20

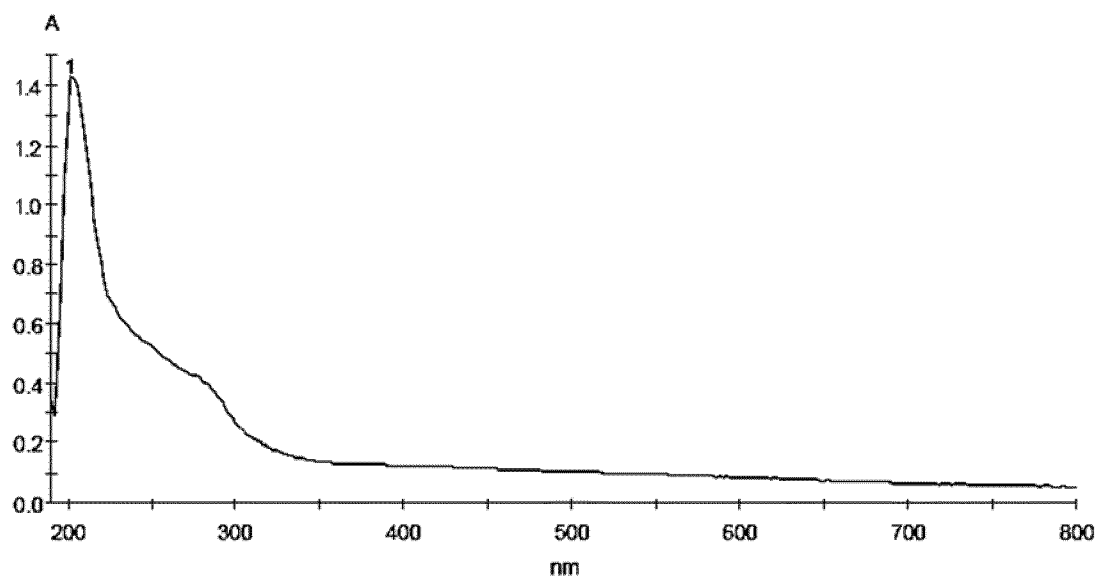


FIG. 21

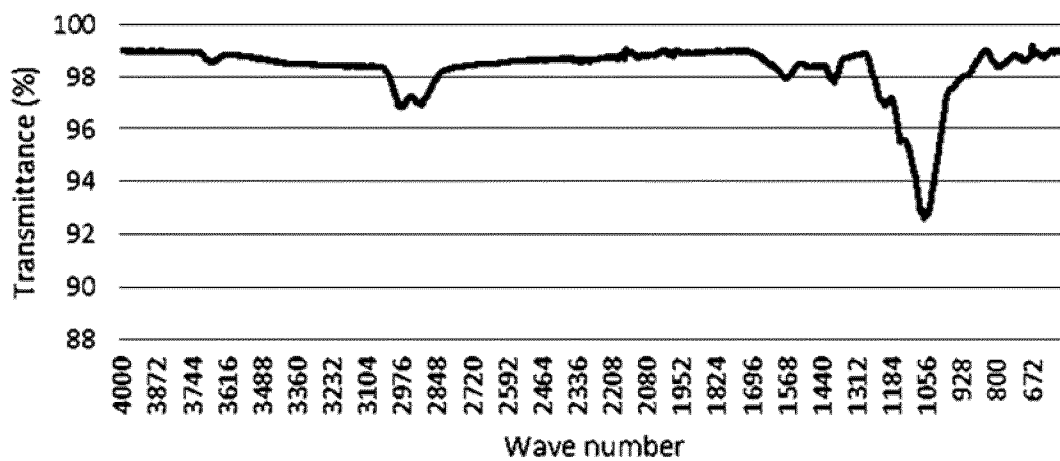


FIG. 22

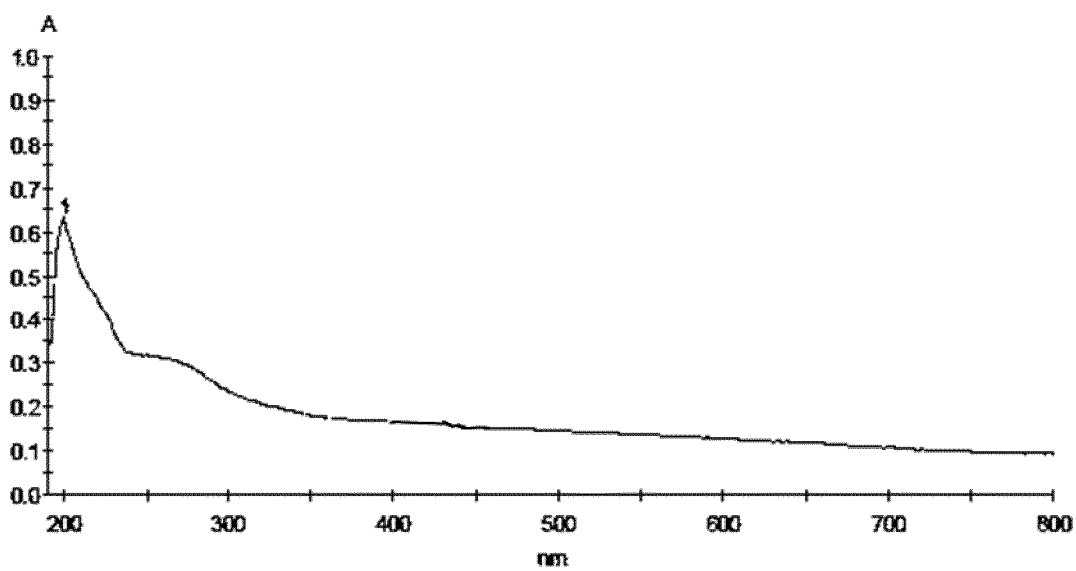


FIG. 23

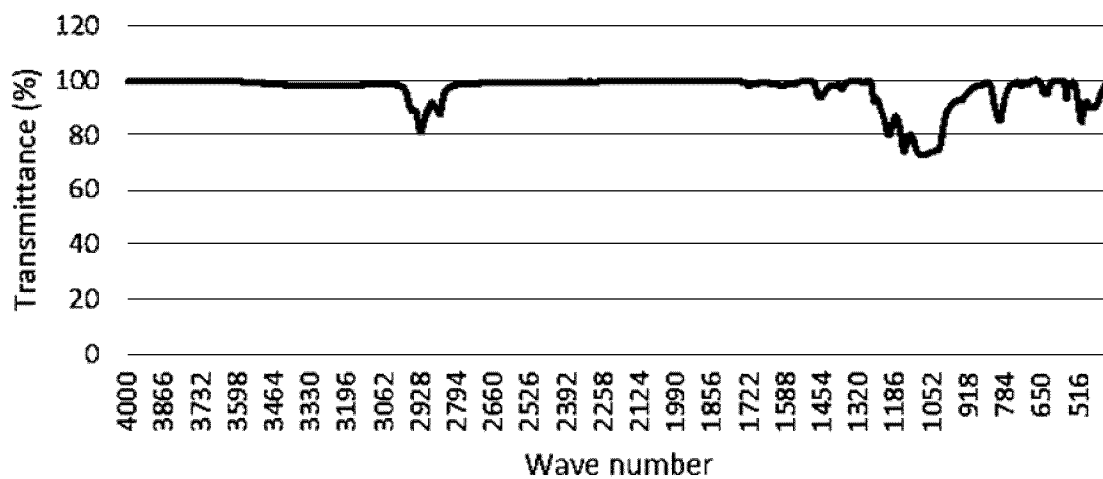


FIG. 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067667

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K49/04 A61K41/00 B22F1/00 B22F9/16 B82Y40/00
B82Y5/00 B82Y15/00 A61L2/16
ADD. C07F9/94

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 C07F B82Y C22C B22F A61L

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, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/170569 A1 (OREGON STATE [US]; GOFORTH ANDREA [US]; BROWN ANNA [US]) 13 December 2012 (2012-12-13)	9-15
Y	page 2, lines 21-27 page 4, lines 5-23 page 6, lines 4-7 page 21, lines 6-23 page 22, lines 15-28 page 23, lines 15-18 page 27, lines 5-13 page 34, lines 7-14 page 36, line 15 - page 37, line 3 page 38, lines 5-25 examples 1,5 claim 2 page 16, lines 6-12 ----- -/--	1-8



Further documents are listed in the continuation of Box C.



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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 September 2019

Date of mailing of the international search report

01/10/2019

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Villard, Anne-Laure

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/067667

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YANG LUO ET AL: "In vitro cytotoxicity of surface modified bismuth nanoparticles", JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE, KLUWER ACADEMIC PUBLISHERS, BO, vol. 23, no. 10, 17 July 2012 (2012-07-17), pages 2563-2573, XP035121187, ISSN: 1573-4838, DOI: 10.1007/S10856-012-4716-1 page 2564, left-hand column, paragraphs 1,2 page 2565, left-hand column, paragraph 2 page 2566, section 2.3 "Surface modifications" -----	7
Y	ANNA L. BROWN ET AL: "Synthesis, X-ray Opacity, and Biological Compatibility of Ultra-High Payload Elemental Bismuth Nanoparticle X-ray Contrast Agents", CHEMISTRY OF MATERIALS, vol. 26, no. 7, 24 March 2014 (2014-03-24), pages 2266-2274, XP055530514, ISSN: 0897-4756, DOI: 10.1021/cm500077z page 2267; figure 1 page 2267, section "Synthesis of BiNPs" page 2269, left-hand column, last paragraph - page 2270, left-hand column, paragraph 2 page 2270; figure 2 page 2273, section "Conclusions" -----	1-8
A	CLAUDIO CABRAL-ROMERO ET AL: "Zerovalent bismuth nanoparticles inhibit Streptococcus mutans growth and formation of biofilm", INTERNATIONAL JOURNAL OF NANOMEDICINE, 1 April 2012 (2012-04-01), page 2109, XP055530063, DOI: 10.2147/IJN.S29854 abstract page 2109, paragraph 1 page 2110, left-hand column, paragraph 3 -----	9-11,13,14
A	STOKES K L ET AL: "Synthesis and properties of bismuth nanocrystals", EIGHTEEN INTERNATIONAL CONFERENCE ON THERMOELECTRICS, 1999, IEEE, PISCATAWAY, NJ, USA, IEEE, US, 29 August 1999 (1999-08-29), pages 374-377, XP032155424, DOI: 10.1109/ICT.1999.843408 ISBN: 978-0-7803-5451-7 page 374, section "Experiment" ----- -/--	9-11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067667

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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