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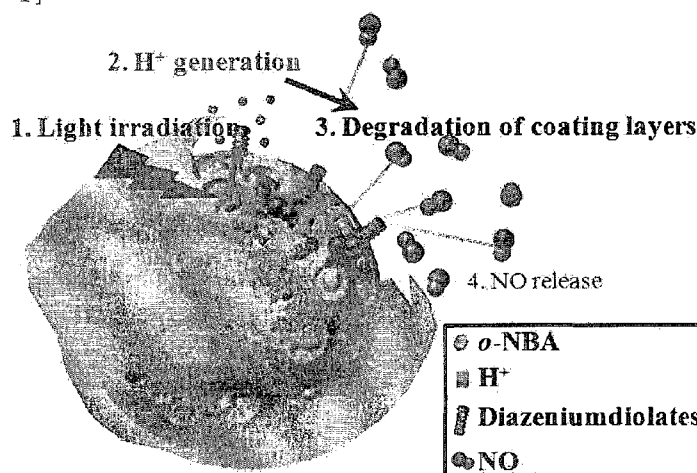
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(54) Title: METHOD OF SELECTIVELY RELEASING NITROGEN MONOXIDE USING MESOPOROUS CORE-SHELL NANOPARTICLE AND CALCIUM PHOSPHATE

[FIG. 1]



(57) Abstract: Provided is a method of controlling release of nitrogen monoxide, and more particularly, a method of selectively releasing nitrogen monoxide using mesoporous silica nanoparticles including a material capable of emitting protons by light irradiation and calcium phosphate. The technique according to the present invention to control release of nitrogen monoxide by light irradiation may stably deliver nitrogen monoxide to a desired part, and induces release of nitrogen monoxide only when irradiated with light, thereby maximizing a therapeutic effect.

## Description

### **Title of Invention: METHOD OF SELECTIVELY RELEASING NITROGEN MONOXIDE USING MESOPOROUS CORE-SHELL NANOPARTICLE AND CALCIUM PHOSPHATE**

#### **Technical Field**

- [1] The present invention relates to a method of controlling release of nitrogen monoxide, and more particularly, to a method of selectively releasing nitrogen monoxide using mesoporous core-shell nanoparticles including a material capable of emitting protons by light irradiation and calcium phosphate.

#### **Background Art**

- [2] Nitrogen monoxide which is a gas synthesized by an intracellular nitrogen monoxide forming enzyme, has an important function as an in vivo physiologically active material. In particular, it is known to be related to various physiological phenomena and diseases such as those in a neurotransmission system, a cardiovascular system and an immune system.
- [3] For example, nitrogen monoxide shows conflicting therapeutic effects in proportion to a concentration and release time. For example, it is known that when nitrogen monoxide with a high concentration is released for a short period of time, an anti-cancer effect and an anti-bacterial effect are generated, and when a small amount of nitrogen monoxide is released for a long period of time, it is related to wound healing, cell growth, angiogenesis and the like. However, since nitrogen monoxide is present in a gas form, there are a lot of constraints on an effective delivery thereof.
- [4] Diazeniumdiolate, a representative functional group releasing nitrogen monoxide is also referred to as NONOate, and may be represented by a general formula of  $RR'N-N(O)=NOR''$ . Diazeniumdiolate may be stably stored in a solid form, has a high solubility in water, decomposes under the condition of in vivo temperature and pH, and also has various release forms depending on the pH. Diazeniumdiolate may easily produce nitrogen monoxide, and release relatively a large amount of nitrogen monoxide, but has a problem of releasing nitrogen monoxide at the same time as contacting water.
- [5] In order to overcome the problem, there has been developed a technique to release nitrogen monoxide by an external stimulus, however, there are very rare cases to use an external stimulus and a biocompatible material. Since a drug delivery system by an external stimulus (light, pH, enzyme, temperature, magnetic field, etc.) has limited selectivity of a drug, it is currently needed to develop a method of stably releasing nitrogen monoxide in vivo.

## **Disclosure of Invention**

### **Technical Problem**

- [6] In order to solve the above problems, an object of the present invention is to provide mesoporous core-shell nanoparticles capable of stably delivering excess nitrogen monoxide to a desired part.
- [7] Further, in order to solve the problem of a diazeniumdiolate functional group which releases nitrogen monoxide at the same time as contacting water, an object of the present invention is to provide a method of selectively releasing nitrogen monoxide by inducing release of nitrogen monoxide only when irradiated with light from the outside, thereby capable of increasing a therapeutic effect, with the mesoporous core-shell nanoparticles of the present invention.

### **Solution to Problem**

- [8] In one general aspect, a mesoporous core-shell nanoparticle includes a mesoporous core portion chemically modified with a silane coupling agent including a secondary amine group; and a shell portion including calcium phosphate, wherein the mesoporous core portion includes a photoreactive pH adjusting agent. Also, a method of preparing the same is disclosed.
- [9] In another general aspect, a method of selectively releasing nitrogen monoxide includes irradiating the mesoporous core-shell nanoparticles with light to release nitrogen monoxide.

### **Advantageous Effects of Invention**

- [10] The method of selectively releasing nitrogen monoxide to which the mesoporous core-shell nanoparticles of the present invention are introduced may stably deliver nitrogen monoxide to a desired part, and induce release of nitrogen monoxide only when irradiated with light, thereby maximizing a therapeutic effect.
- [11] In addition, to the mesoporous core-shell nanoparticles according to the present invention, another drug may be further added, and it is expected that a therapeutic effect of nitrogen monoxide may be generated together with a therapeutic effect of a drug, via dual delivery.

### **Brief Description of Drawings**

- [12] The above and other objects, features and advantages of the present invention will become apparent from the following description of preferred embodiments given in conjunction with the accompanying drawings, in which:
- [13] FIG. 1 illustrates a mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention.
- [14] FIG. 2 illustrates a preparation process of the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention.

- [15] FIG. 3 is a result of measuring the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention with a transmission electron microscopy.
- [16] FIG. 4 is a crystal structure analysis graph of the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention measured via powder XRD.
- [17] FIG. 5 is a result of measuring pH of the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention before and after being irradiated with light.
- [18] FIG. 6 is a result of measuring the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention with an infrared spectroscope (FT-IR).
- [19] FIG. 7 is a result of measuring the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention with a UV-vis spectroscope.
- [20] FIG. 8 is a result of measuring the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention via a calcium ion detection method.
- [21] FIG. 9, 10 are the results of measuring the mesoporous core-shell nanoparticle according to an exemplary embodiment of the present invention via a nitrogen monoxide detector.

### **Best Mode for Carrying out the Invention**

- [22] Hereinafter, the embodiments of the present invention will be described in detail with reference to accompanying drawings. Technical terms and scientific terms used in the present specification have the general meaning understood by those skilled in the art to which the present invention pertains unless otherwise defined, and a description for the known function and configuration obscuring the gist of the present invention will be omitted in the following description.
- [23] The present invention relates to a mesoporous core-shell nanoparticle including:
- [24] a mesoporous core portion chemically modified with a silane coupling agent including a secondary amine group; and
- [25] a shell portion including calcium phosphate,
- [26] wherein the mesoporous core portion includes a photoreactive pH adjusting agent.
- [27] Among the terms used herein, a mesoporous core-shell nanoparticle refers to a mesoporous core-shell nanoparticle having uniform sized pores and arrangement of the pores in the structure. Further, photoreactive refers to a chemical bonding state being changed with light irradiation, and a light source of the present invention may be an ultraviolet ray, preferably an ultraviolet ray having a wavelength of 200 to 400 nm. Further, a photoreactive pH adjusting agent refers to a substance capable of adjusting

pH by producing protons by light irradiation.

[28] Hereinafter, the mesoporous core-shell nanoparticle of the present invention will be described in detail.

[29] The mesoporous core portion may include any one selected from silica (SiO<sub>2</sub>), alumina (Al<sub>2</sub>O<sub>3</sub>), titania (TiO<sub>2</sub>), zirconia (ZrO<sub>2</sub>), antimony trioxide (Sb<sub>2</sub>O<sub>3</sub>), molybdenum oxide (MoO<sub>3</sub>), tin oxide (SnO<sub>2</sub>), zinc oxide (ZnO), and mixtures thereof, and preferably include silica (SiO<sub>2</sub>), but is not limited thereto.

[30] The mesoporous core-shell nanoparticles of the present invention may have a average diameter of 30 - 200 nm, but are not limited thereto.

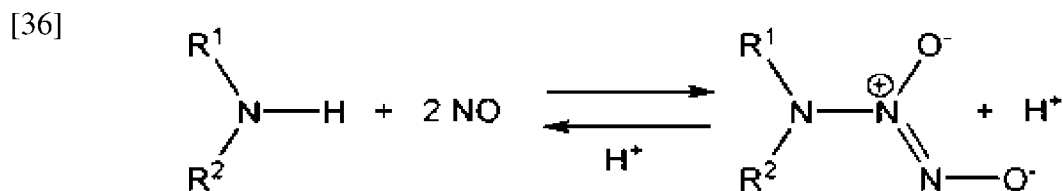
[31] The mesoporous core-shell nanoparticles of the present invention may capture substances having various sizes, shapes and functions, and the biocompatible pores within the structure may capture foreign substances with excellent stability. Therefore, the mesoporous core-shell nanoparticles may be applied to drug and gene deliveries, cell imaging and cancer treatment.

[32] Particularly, since the mesoporous core-shell nanoparticles of the present invention are easily surface-functionalized, mesoporous core-shell nanoparticles which are surface-functionalized so as to be applied to stimulus-controlled release are introduced.

[33] In the present invention, a functional group capable of releasing nitrogen monoxide was introduced to the mesoporous core-shell nanoparticles, and the functional group may be "diazoniumdiolate".

[34] The diazeniumdiolate functional group may be obtained by reacting a secondary amine with nitrogen monoxide, as represented by the following Reaction Formula 1. Further, the diazeniumdiolate functional group may be represented by a general formula of RR'N-N(O)=NOR'.

[35] [Reaction Formula 1]

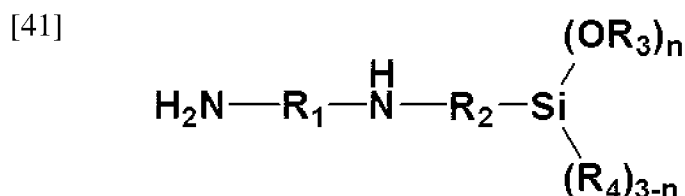


[37] Since the diazeniumdiolate functional group releases two molecules of nitrogen monoxide per the functional group, it may generate nitrogen monoxide with a relatively high concentration, when it is included in a carrier. However, since it releases nitrogen monoxide at the same time as contacting water, there is needed a gatekeeper system controlling release of nitrogen monoxide via an external stimulus for efficient nitrogen monoxide delivery. Therefore, in the present invention, the diazeniumdiolate functional group may be reacted to release nitrogen monoxide, when exposed to a certain condition in vivo.

[38] The present invention may use a silane coupling agent including a secondary amine group capable of forming a diazeniumdiolate functional group. An alkoxysilyl (Si-OR) functional group of the silane coupling agent may become a silanol group when hydrolyzed to be bound to an inorganic material, thereby serving to connect an organic material and an inorganic material to each other.

[39] The silane coupling agent may be represented by the following Chemical Formula 1:

[40] [Chemical Formula 1]



[42] wherein

[43]  $\text{R}_1$  and  $\text{R}_2$  are independently of each other a (C1-C12) alkylene group;  $\text{R}_3$  and  $\text{R}_4$  are independently of each other a (C1-C4) alkyl group; and  $n$  is 1-3.

[44] In the present invention, more preferably, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEATS) may be used to obtain a desired effect of the present invention, but not limited thereto.

[45] The present invention may control release of nitrogen monoxide by changing the pH, by using a photoreactive pH adjusting agent and calcium phosphate as a gatekeeper system controlling release of nitrogen monoxide. The calcium phosphate used in the present invention is coated on a shell of the mesoporous core-shell nanoparticle, and when the pH is lowered, calcium phosphate coated layer decomposes, thereby selectively releasing nitrogen monoxide.

[46] The photoreactive pH adjusting agent used in the present invention which is a material producing protons by light irradiation, may be any one selected from the group consisting of o-nitrobenzaldehyde (o-NBA), m-nitrobenzaldehyde (m-NBA), p-nitrobenzaldehyde (p-NBA), and mixtures thereof. Preferably o-NBA may be used, and o-NBA which is a compound releasing protons upon exposure to UV having a certain wavelength, is changed to a nitrosobenzoic anion via an intramolecular proton transfer reaction in an excited state, and finally releases protons. Therefore, the pH changes rapidly by a photoreaction, and such pH change may decompose the calcium phosphate coated layer.

[47] The calcium phosphate used in the present invention may refer to phosphates of calcium, and preferably hydroxyapatite may be used.

[48] The Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is a biological inorganic compound, and has the most similar structure to human bones, and thus, when it is bound to polymer, it has excellent bone regeneration ability, bioactivity, biocompatibility and

biodegradability as a soft tissue substitute, thereby being mainly utilized in tissue engineering. In the present invention, the hydroxyapatite may be used as a gatekeeper system controlling effective release of nitrogen monoxide, using its property of being decomposed with lowered pH.

[49] The present invention may provide a method of preparing mesoporous core-shell nanoparticles including:

[50] a) treating mesoporous nanoparticles with a silane coupling agent;

[51] b) adding a photoreactive pH adjusting agent to the mesoporous nanoparticles prepared in a);

[52] c) coating a surface of the mesoporous nanoparticles prepared in b) with calcium phosphate; and

[53] d) adding nitrogen monoxide gas to the mesoporous nanoparticles prepared in c), thereby forming a diazeniumdiolate functional group.

[54] a) Treating the mesoporous nanoparticles with a silane coupling agent is as follows:

[55] The method of preparing mesoporous nanoparticles of the present invention may be carried out by a person skilled in the art with a common method, and in the present invention, by adding a surfactant to a metal precursor. The metal precursor may be preferably a silica precursor represented by  $\text{Si}(\text{OR})_4$  (R is independently of each other hydrogen or a (C1-C4) alkyl group), for example, tetraethyl orthosilicate (TEOS), tetramethyl orthosilicate (TMOS), tetrapropyl orthosilicate (TPOS), and the like, but not limited thereto.

[56] The surfactant may include one or more selected from the group consisting of a cationic surfactant, an anionic surfactant, an amphoteric surfactant, and a non-ionic surfactant.

[57] As the cationic surfactant, an alkyltrimethylammonium salt, an alkylpyridinium salt, an alkyl quaternary ammonium salt, an alkyl dimethylbenzylammonium salt, and the like may be used, and as the anionic surfactant, an alkylsulfate salt, an alkylsulfonate salt, an alkylphosphate salt, an alkylcarboxylate salt, and the like may be used.

[58] As the amphoteric surfactant, cocoamido propyl betaine, cocoamphoacetate, cocoamphocarboxyglycinate, alkyl amphoacetate, sodium lauroamphoacetate, and the like may be used.

[59] The non-ionic surfactant may be a sorbitan-based surfactant, a sugar-based surfactant, or a poly(alkylene oxide)-based surfactant. The sugar-based surfactant may be alkyl polyglucoside, and as the sugar, a monosaccharide or a disaccharide may be used, and sucrose distearate, sucrose monostearate and the like may be used. The poly(alkylene oxide)-based surfactant may be alkyl or aryl-substituted poly(alkylene oxide), or a hydrophilic poly(alkylene oxide)-hydrophobic poly(alkylene oxide)-type block copolymer, and specifically, poly(ethylene oxide)-stearate, nonylphenol

poly(ethylene oxide), ethylene oxide-added sorbitan fatty acid ester, a poly(ethylene oxide)-poly(propylene oxide) block copolymer, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer, and the like may be used.

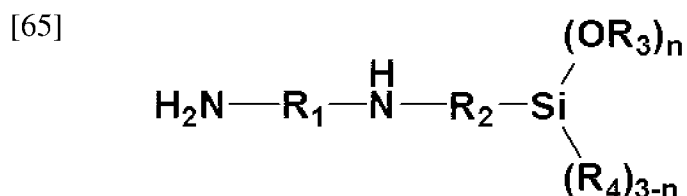
[60] In the present invention, preferably a cationic surfactant consisting of a hydrophobic alkyl chain and hydrophilic amine may be used, and hexadecyltrimethylammonium bromide (CTAB), hexadecyltrimethylammonium chloride (CTACl) and the like may be used, but are not limited thereto.

[61] The mesoporous nanoparticles may be prepared by centrifuging a mixture of the surfactant, the silica precursor and a solvent.

[62] The solvent of the present invention may be alcohols, water, an ether-based solvent alone or in combination, and the alcohol solvent may be methanol, ethanol, iso-propanol, propanol, butanol, pentanol, and the like, and the ether-based solvent may be tetrahydrofuran, methyltetrahydrofuran, dimethylether, dibutylether, and the like, but not limited thereto.

[63] With the prepared mesoporous nanoparticles, a silane coupling agent of the following Chemical Formula 1 may be mixed:

[64] [Chemical Formula 1]



[66] wherein

[67]  $\text{R}_1$  and  $\text{R}_2$  are independently of each other a (C1-C12) alkylene group;  $\text{R}_3$  and  $\text{R}_4$  are independently of each other a (C1-C4) alkyl group; and  $n$  is 1-3.

[68] Further, in step a), the surfactant may be removed from the prepared mesoporous nanoparticles. In order to remove the surfactant, the mesoporous nanoparticles may be immersed in a mixed solution of hydrochloric acid and methanol.

[69] b) Adding the photoreactive pH adjusting agent to the mesoporous nanoparticles prepared in step a) is as follows:

[70] Mesoporous nanoparticle powder prepared in step a) and a liquid photoreactive pH adjusting agent may be mixed and dried. The photoreactive pH adjusting agent used in the present invention may be any one selected from the group consisting of o-nitrobenzaldehyde, m-nitrobenzaldehyde, p-nitrobenzaldehyde, and mixtures thereof.

[71] c) Coating the surface of the mesoporous nanoparticles prepared in step b) with calcium phosphate is as follows:

[72] The calcium phosphate used in the present invention may be hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), and as a preparation method of the hydroxyapatite, a wet process of

preparing a water-soluble calcium salt and a phosphate using a liquid medium, but not limited thereto.

[73] The hydroxyapatite of the present invention is sensitive to pH when reacted, and forms precipitates at about pH 12, but as the reaction proceeds, pH is lowered, and thus, at the end of the reaction, may form precipitates in a range of pH 7 to 11.

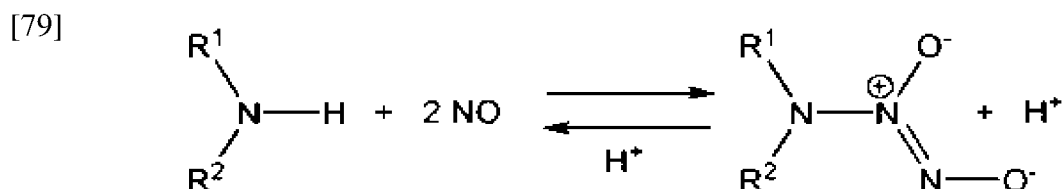
[74] Since the hydroxyapatite precipitates may not secure sufficient calcium within their crystal structure in a low pH range less than pH 7, thereby producing hydroxyapatite lacking calcium, that is, structurally collapsed hydroxyapatite.

[75] In the present invention, pH may be adjusted to 7 to 11.

[76] d) Adding nitrogen monoxide gas to the mesoporous nanoparticles prepared in step c), thereby forming a diazeniumdiolate functional group, is as follows:

[77] The diazeniumdiolate functional group may be obtained by reacting a secondary amine with nitrogen monoxide gas, as represented by the following Reaction Formula 1.

[78] [Reaction Formula 1]



[80] In the present invention, the nitrogen monoxide gas may be added under a pressure of 40 psi to 200 psi, preferably under a pressure of 80 psi to 150 psi, but is not limited thereto.

[81] The mesoporous core-shell nanoparticles of the present invention as prepared above may be irradiated with light to selectively release nitrogen monoxide, and the light irradiation uses an ultraviolet ray at a wavelength of 200 to 400 nm.

[82] In addition, the mesoporous core-shell nanoparticles of the present invention may further include a pharmacologically effective material within or on the surface of the particles. They may be used for the purpose of an additional pharmacological effect, together with the nitrogen monoxide effect of the present invention. The pharmacologically effective material is not limited, but may include preferably a material preventing thrombopoiesis or blood clots, an antioxidant, an anti-inflammatory agent, a wound healing promoting material, antibacterial agent, and the like, and the therapeutic effect of nitrogen monoxide may be generated together with the therapeutic effect of the drug.

[83] Hereinafter, the present invention will be described in more detail by the following Examples. However, the following Examples are only a reference for describing the present invention in detail, and the present invention is not limited thereto.

[84]

[85] [Example 1]

[86] 1. Reaction of mesoporous silica nanoparticles and a silane coupling agent (MSN-AEATS)

[87] 3.5 ml of a 2M NaOH solution was added to a solution of 1 g of cetyltrimethylammonium bromide (CTAB) dissolved in 500 ml of distilled water. After stirring for 15 minutes, 5 ml of tetraethyl orthosilicate (TEOS) was added. After stirring at 80 °C for 3 hours, the solution was filtered, washed three times with MeOH, and dried. 0.5 g of the dried powder, 100 ml of EtOH, and 0.5 ml of N-(2-aminoethyl)-3-aminopropyl trimethoxysilane (AEATS) were added to be refluxed with a suspension reaction for 3 hours. After being separated by a centrifuge (7000 rpm, 10 min), the reactants were washed three times with MeOH. The reactants were dissolved in a mixed solution of 16 ml of MeOH and 1 ml of concentrated hydrochloric acid at 60 °C for 24 hours, and dried. Through the process, mesoporous silica nanoparticles (MSN-AEATS) in which CTAB is dissolved to be mixed with AEATS were obtained.

[88] 2. Reaction of a photoreactive pH adjusting agent (pH@MSN-AEATS)

[89] 100 mg of the obtained MSN-AEATS was mixed with 10 ml of 2-nitrobenzaldehyde (o-NBA), and left at a room temperature for 24 hours. The mixture was freeze-dried for 48 hours to obtain pH@MSN-AEATS.

[90] 3. Reaction with calcium phosphate (pH@MSN-CaP)

[91] 100 mg of the obtained pH@MSN-AEATS was dissolved in 10 ml of a 0.1M (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solution, and then ammonia water was added thereto, thereby adjusting pH to 10. 20 ml of 0.1M Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O was added by dropwise, and reacted at 60 °C for 1 hour. After being separated by a centrifuge (7000 rpm, 10 min), washing was carried out three times with deionized water. The reactants were freeze-dried for 48 hours to obtain pH@MSN-AEATS.

[92] 4. Reaction of a diazeniumdiolate functional group (pH@MSN-CaP-NO)

[93] 10 mg of the obtained pH@MSN-CaP was dissolved in 3 ml of 0.5 M NaOMe/MeOH, and then added to a high pressure reactor. The reactor was purged twice with 20 psi of Ar gas and then reaction was carried out with 80 psi of NO gas for three days. The pH@MSN-CaP-NO having a diazeniumdiolate group capable of releasing nitrogen monoxide was separated in a centrifuge (7000 rpm, 10 min), and then the remaining solvent was removed by vacuum drying.

[94] [Characteristic evaluation]

[95] Nitrogen monoxide release behavior of the mesoporous silica nanoparticles before and after light irradiation with UV at 365 nm (300 μW/cm<sup>2</sup>) was observed.

[96] (1) Confirmation of mesoporous silica nanoparticles

[97] In order to confirm mesoporous silica nanoparticles, a transmission electron mi-

croscopy (JEOL JEM-1011, Japan) was used for observation. As a result of observing the size and distribution through FIG. 3, it was confirmed that the nanoparticles were uniformly formed in a size of about 4-50 nm, and when exposed to UV, a calcium phosphate layer on the surface thereof collapsed.

[98] (2) Crystal structure analysis

[99] The surface of the mesoporous silica nanoparticles was observed through powder XRD (using Cu K $\alpha_1$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) from Rigaku SmartLab at 40 kV and 30 mA). As a result of confirmation through the powder XRD, a specific peak which occurs only in mesoporous silica nanoparticles, and a reduced surface area due to calcium phosphate were shown, and there was no change in the crystal structure by surface modification. The results are illustrated in FIG. 4.

[100] (3) Confirmation whether a pH adjusting agent is operated by UV

[101] As a result of irradiating o-NBA with UV in order to confirm that pH is changed by UV, it was confirmed that pH was changed from 8 to 5, and as a result of actually carrying out the same method by adding a pH adjusting agent to mesoporous silica particles, it was confirmed that pH was lowered similarly to o-NBA. In the case of being coated with calcium phosphate, it was confirmed that a calcium phosphate layer collapsed with lowered pH, which caused phosphate ions to be produced ( $pK_b \sim 10^{-2}$ ), thereby increasing the pH. The results are illustrated in FIG. 5.

[102] (4) Confirmation of nitrogen monoxide through an infrared spectroscope (FT-IR) and a UV-vis spectroscope

[103] As a result of observing the mesoporous silica nanoparticles through an infrared spectroscope and a UV-vis spectroscope by each step of surface modification, a specific peak formation due to nitrogen monoxide was shown, thereby confirming release of nitrogen monoxide. As a result of UV-vis spectroscopic observation, an absorption band of diazeniumdiolate was recorded as 250-260 nm. The results are illustrated in FIGS. 6 and 7.

[104] (5) Calcium ion detection by UV (Arsenazo assay)

[105] (Arsenazo III complexation method)[Micaylova V. Et al., Anal. Chim. Acta, 53(194), 1971]

[106] In order to confirm that a calcium phosphate layer on the surface collapses by operating the pH adjusting agent by UV, the amount of calcium ion detected by leaving the particles under UV for 1 hour was measured. When not being exposed to UV, 6.4% calcium ions were detected, however, when being irradiated with light, 56% calcium ions were detected, thereby confirming the operation of the pH adjusting agent. The results are illustrated in FIG. 8.

[107] (6) Nitrogen monoxide detector (SieversNOA)

[108] As a result of confirming nitrogen monoxide release control by light irradiation using

a nitrogen monoxide detector (SieversNOA, GE analytical instruments, USA), nitrogen monoxide was hardly released, when not irradiated with UV, however, a highest release point of nitrogen monoxide was increased to 25 times or more, and a total release amount was also increased to 10 times or more, when irradiated with UV. Further, as a result of carrying out an experiment depending on on/off of UV, it was confirmed that nitrogen monoxide was released only when the UV is on. The results are illustrated in FIG. 9.

[109] Hereinabove, although the present invention has been described by specific matters, exemplary embodiments, and drawings, they have been provided only for assisting in the entire understanding of the present invention. Therefore, the present invention is not limited to the exemplary embodiments. Various modifications and changes may be made by those skilled in the art to which the present invention pertains from this description.

[110] Therefore, the spirit of the present invention should not be limited to the above-described exemplary embodiments, and the following claims as well as all modified equally or equivalently to the claims are intended to fall within the scope and spirit of the invention.

## Claims

- [Claim 1] A mesoporous core-shell nanoparticle comprising:  
a mesoporous core portion chemically modified by a silane coupling agent including a secondary amine group; and  
a shell portion including calcium phosphate,  
wherein the mesoporous core portion includes a photoreactive pH adjusting agent.
- [Claim 2] The mesoporous core-shell nanoparticle of claim 1, wherein the mesoporous core portion includes any one selected from the group consisting of silica (SiO<sub>2</sub>), alumina (Al<sub>2</sub>O<sub>3</sub>), titania (TiO<sub>2</sub>), zirconia (ZrO<sub>2</sub>), antimony trioxide (Sb<sub>2</sub>O<sub>3</sub>), molybdenum oxide (MoO<sub>3</sub>), tin oxide (SnO<sub>2</sub>), zinc oxide (ZnO), and mixtures thereof.
- [Claim 3] The mesoporous core-shell nanoparticle of claim 1, wherein the silane coupling agent is represented by following Chemical Formula 1:  
[Chemical Formula 1]
- $$\text{H}_2\text{N}-\text{R}_1-\overset{\text{H}}{\underset{|}{\text{N}}}-\text{R}_2-\text{Si}\begin{matrix} \nearrow (\text{OR}_3)_n \\ \searrow (\text{R}_4)_{3-n} \end{matrix}$$
- wherein  
R<sub>1</sub> and R<sub>2</sub> are independently of each other a (C1-C12) alkylene group;  
R<sub>3</sub> and R<sub>4</sub> are independently of each other a (C1-C4) alkyl group; and n is 1-3.
- [Claim 4] The mesoporous core-shell nanoparticle of claim 1, wherein the silane coupling agent includes a diazeniumdiolate functional group formed by reaction of the secondary amine group with nitrogen monoxide.
- [Claim 5] The mesoporous core-shell nanoparticle of claim 1, wherein the photoreactive pH adjusting agent is any one selected from the group consisting of o-nitrobenzaldehyde, m-nitrobenzaldehyde, p-nitrobenzaldehyde, and mixtures thereof.
- [Claim 6] The mesoporous core-shell nanoparticle of claim 1, wherein the calcium phosphate is hydroxyapatite (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>).
- [Claim 7] A method of preparing mesoporous core-shell nanoparticles comprising:  
a) treating mesoporous nanoparticles with a silane coupling agent;  
b) adding a photoreactive pH adjusting agent to the mesoporous nanoparticles prepared in a);

c) coating a surface of the mesoporous nanoparticles prepared in b) with calcium phosphate; and

d) adding nitrogen monoxide gas to the mesoporous nanoparticles prepared in c), thereby forming a diazeniumdiolate functional group.

[Claim 8] The method of claim 7, wherein the mesoporous nanoparticles in a) are formed by adding a surfactant to a metal precursor.

[Claim 9] The method of claim 8, wherein the metal precursor includes  $\text{Si}-(\text{OR})_4$  (R is independently of each other hydrogen or a (C1-C4) alkyl group).

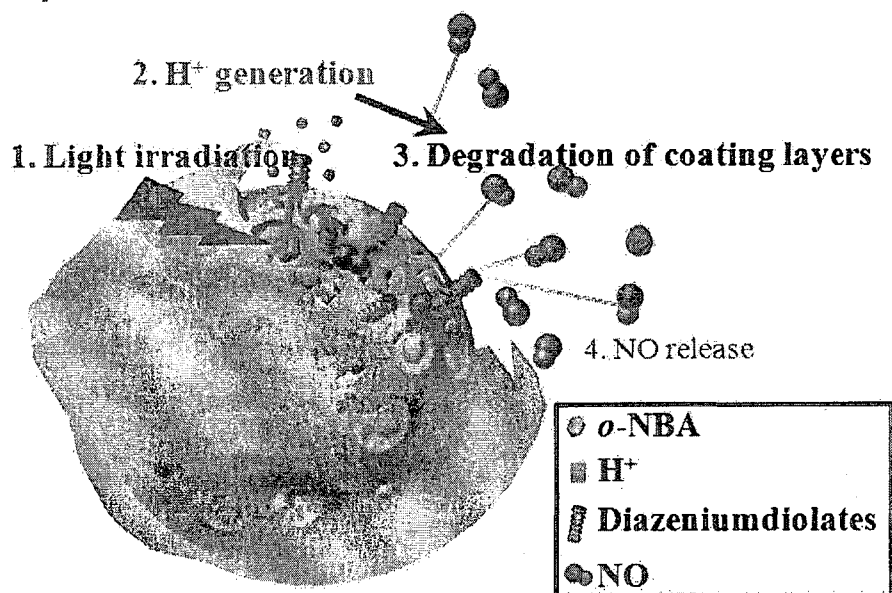
[Claim 10] The method of claim 8, wherein the surfactant includes one or more selected from the group consisting of a cationic surfactant, an anionic surfactant, an amphoteric surfactant, and a non-ionic surfactant.

[Claim 11] The method of claim 7, wherein pH is adjusted to 7 to 11 in c).

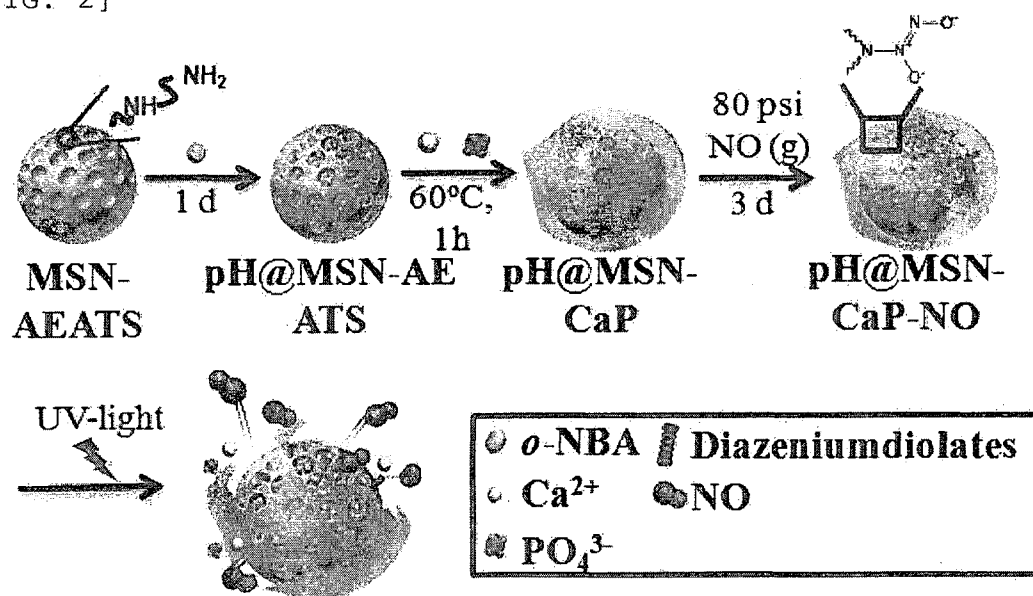
[Claim 12] A method of selectively releasing nitrogen monoxide, wherein the nitrogen monoxide is released by irradiating the mesoporous core-shell nanoparticles of any one of claims 1 to 6 with light.

## [Drawings]

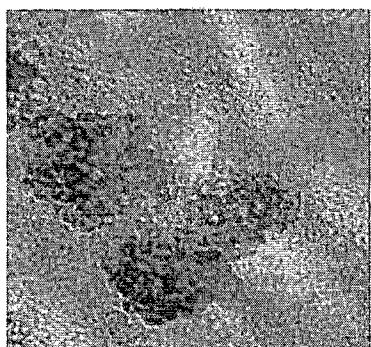
[FIG. 1]



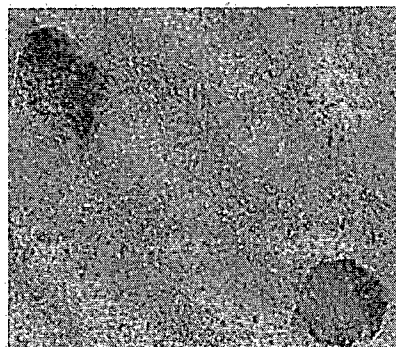
[FIG. 2]



[FIG. 3]

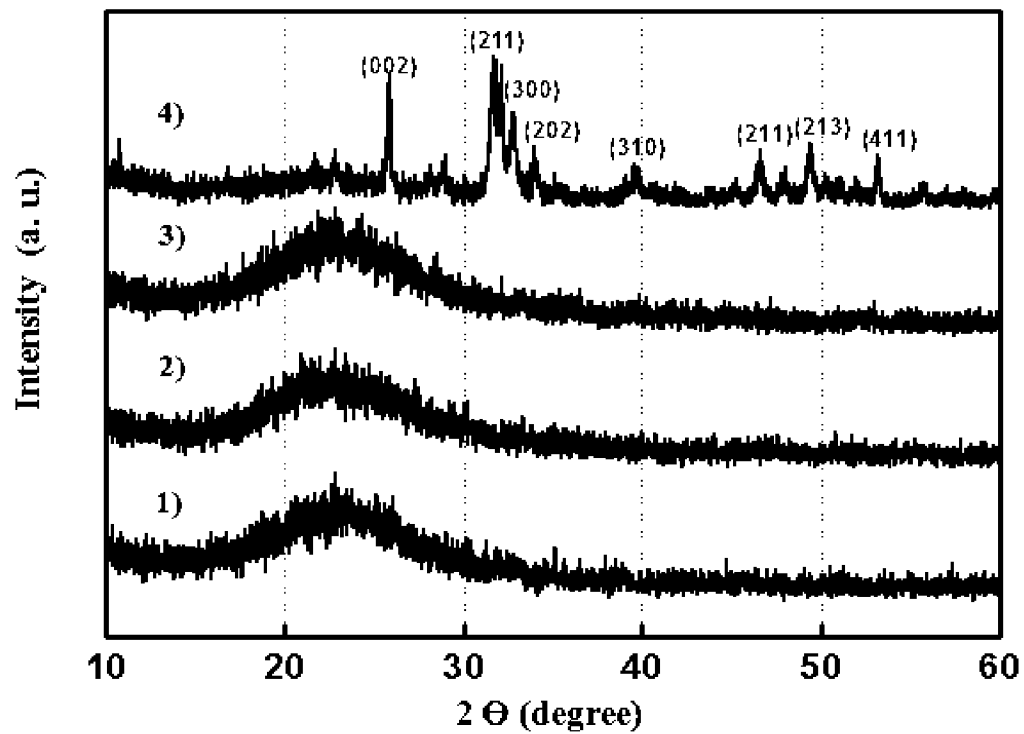


(a)Before UV



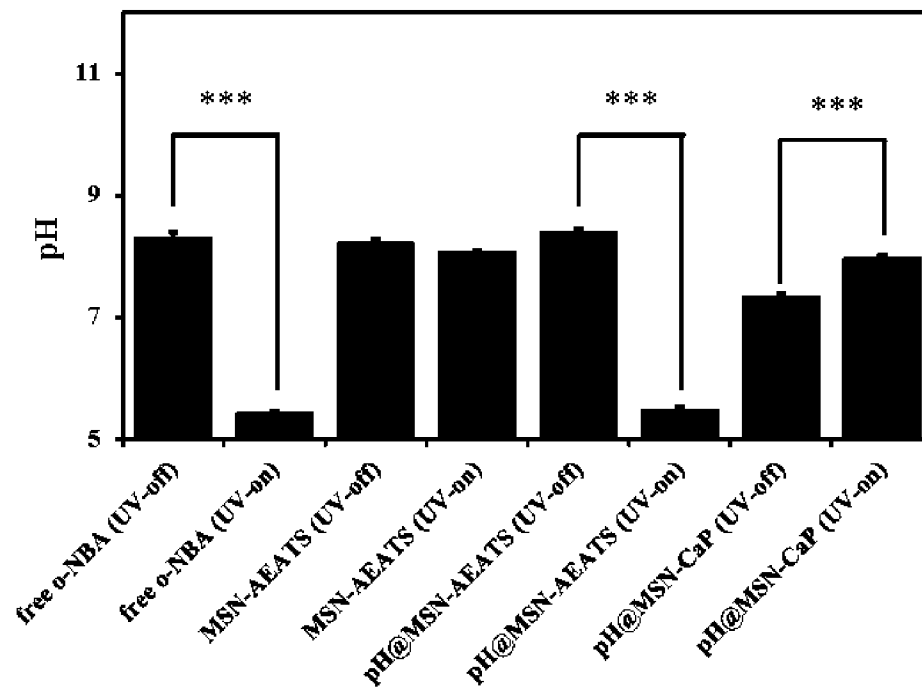
(b)After UV

[Fig. 4]

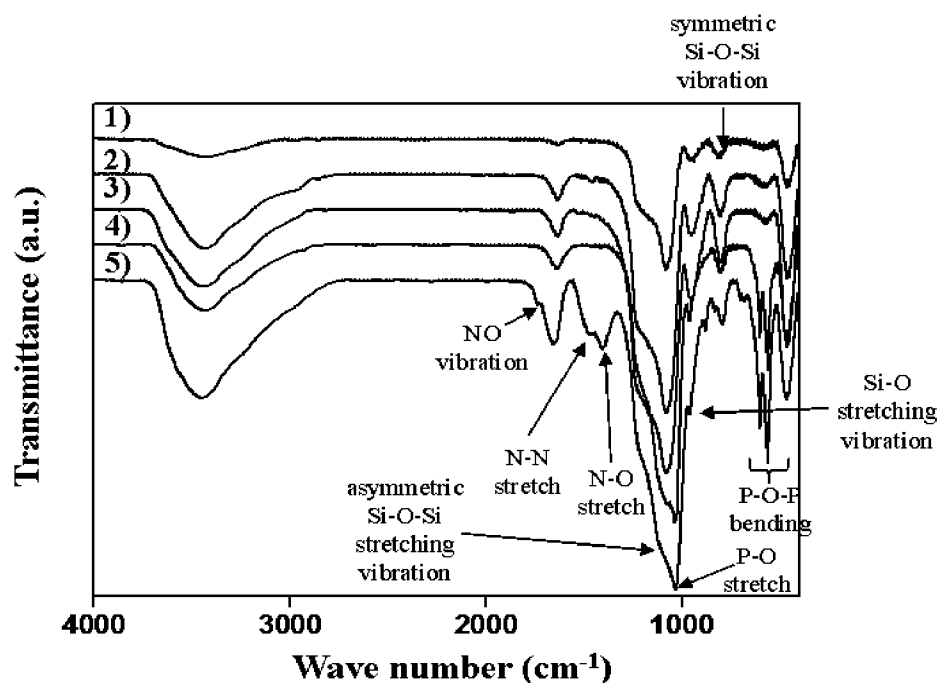


(1) Pure MSN, 2) MSN-AEATS, 3) pH@MSN-AEATS, 4) pH@MSN-CaP,

[Fig. 5]

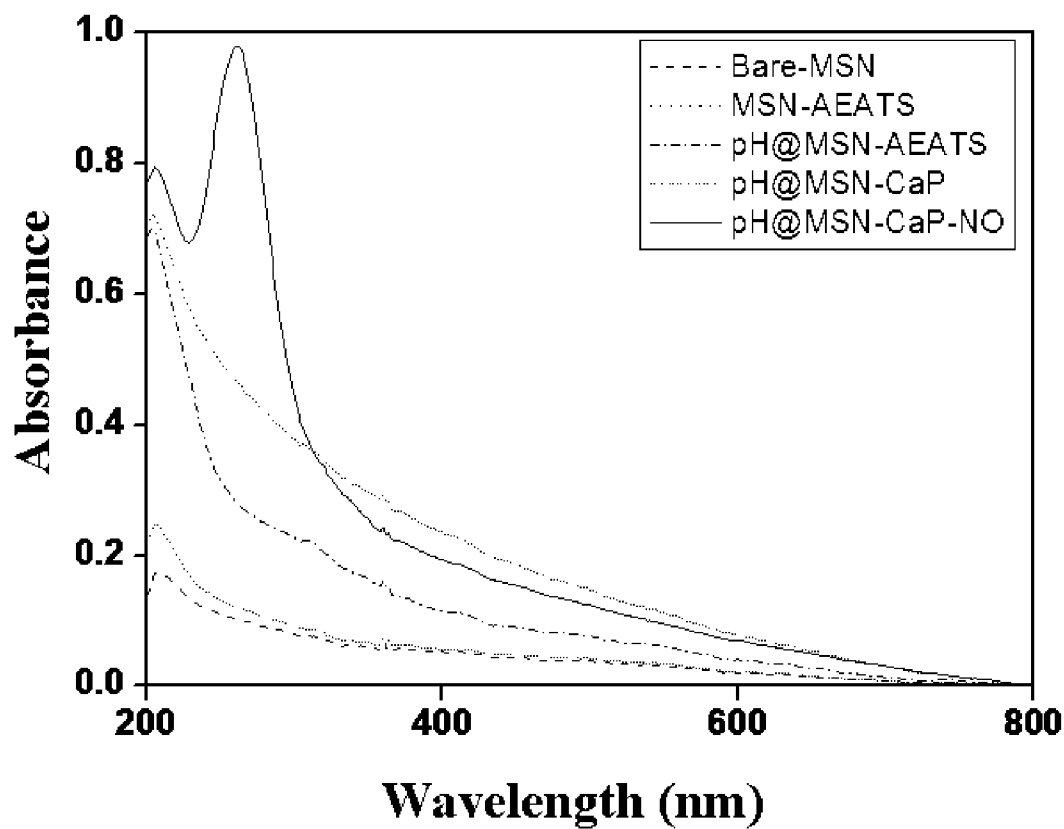


[Fig. 6]

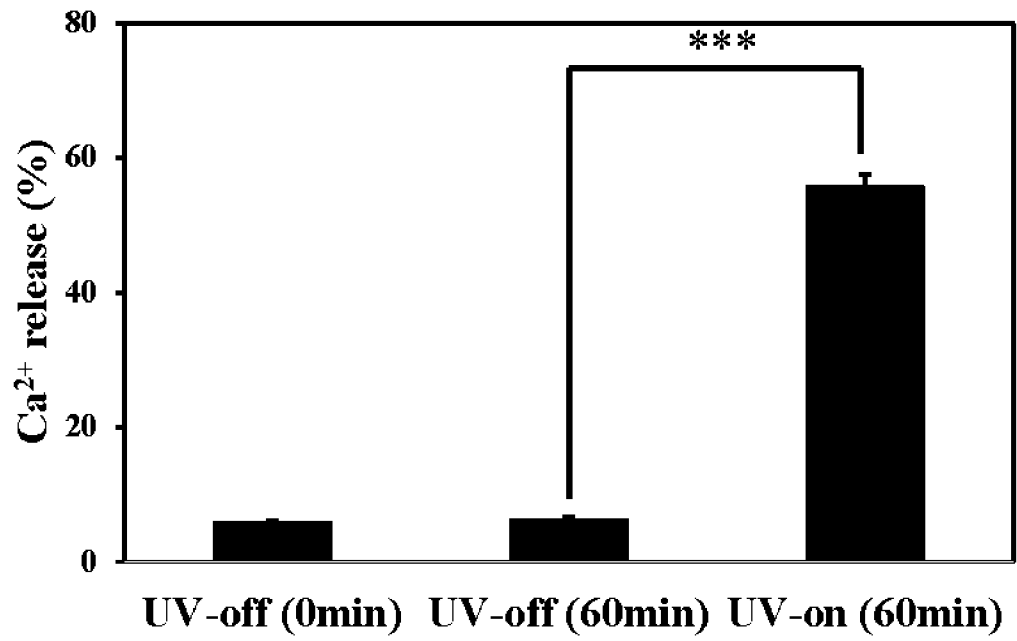


- 1) Pure MSN, 2) MSN-AEATS, 3) pH@MSN-AEATS,  
4) pH@MSN-CaP, 5) pH@MSN-CaP-NO

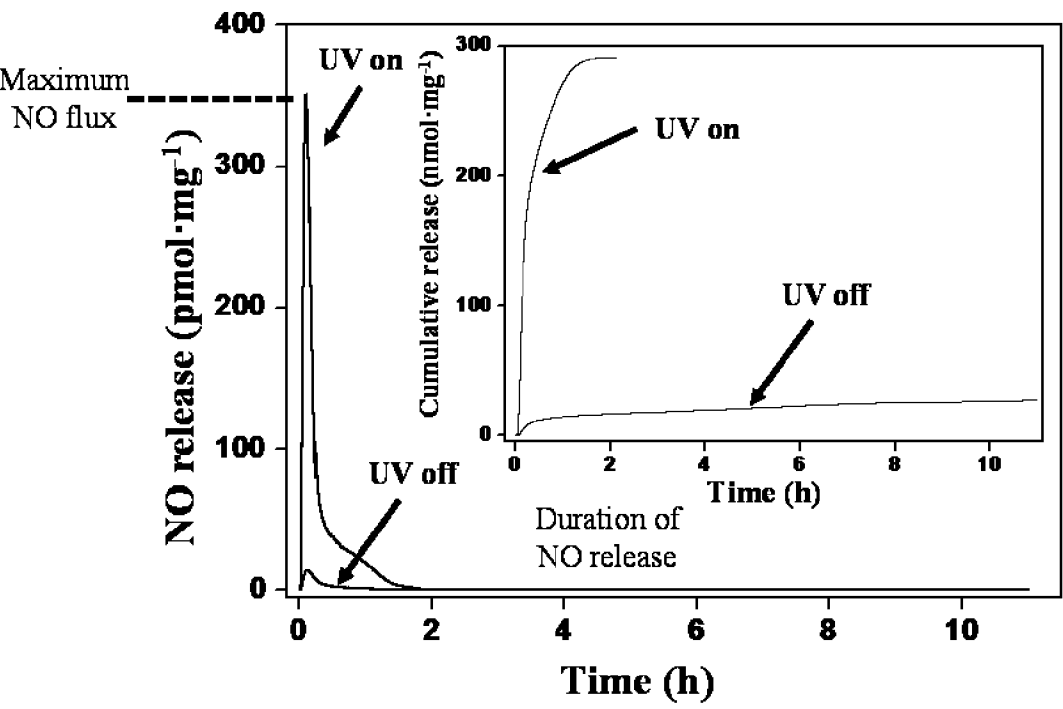
[Fig. 7]



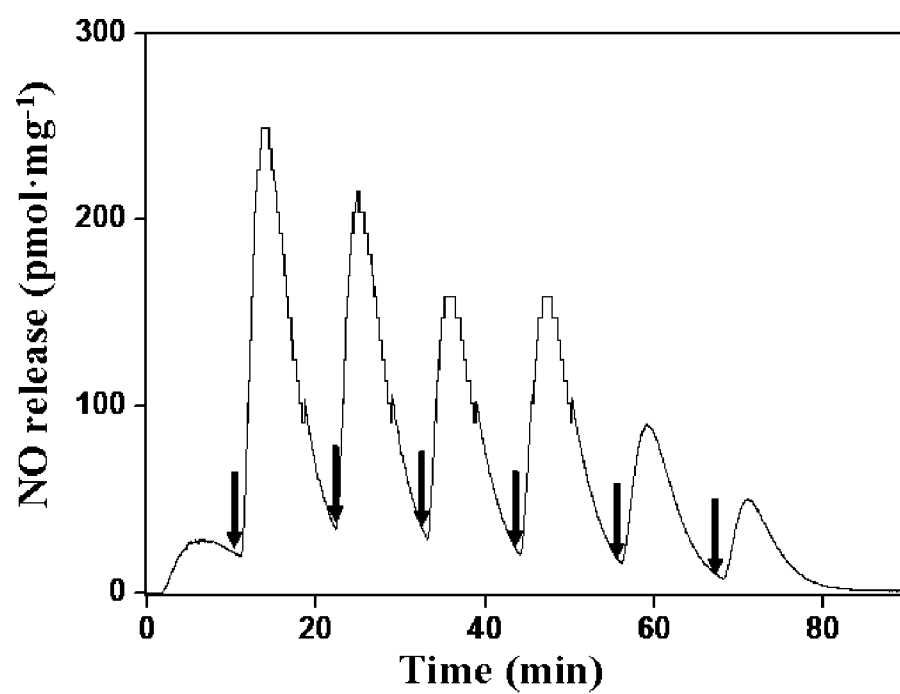
[Fig. 8]



[Fig. 9]



[Fig. 10]



**A. CLASSIFICATION OF SUBJECT MATTER****A61K 9/14(2006.01)i, A61K 33/00(2006.01)i, A61K 41/00(2006.01)i**

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 9/14; A61K 9/00; C07F 7/10; A61K 33/00; A61K 41/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

**eKOMPASS(KIPO internal) & Keywords: nanoparticle, core, shell, calcium phosphate, nitrogen monoxide, diazeniumdiolate, nonoate****C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                   | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | KIM, J. et al., "A platform for nitric oxide delivery", Journal of Materials Chemistry B, 2014, Vol. 2, No. 4, pp. 341-356.<br>See abstract; and page 346, left column.                                                                                              | 1-12                  |
| A         | RIM, H. P. et al., "pH-Tunable Calcium Phosphate Covered Mesoporous Silica Nanocontainers for Intracellular Controlled Release of Guest Drugs", Angewandte Chemie International Edition, 2011, Vol. 50, No. 38, pp. 8853-8857<br>See pages 8853, 8854; and figure 1. | 1-12                  |
| A         | KOBAYASHI, K. et al., "Surface engineering of nanoparticles for therapeutic applications", Polymer Journal, 2014, Vol. 46, No. 8, pp. 460-468<br>See page 463; and figure 6.                                                                                         | 1-12                  |
| A         | US 2014-0058124 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 27 February 2014<br>See claim 57.                                                                                                                                                               | 1-12                  |
| A         | US 2011-0151000 A1 (SCHULTZ, W. J. et al.) 23 June 2011<br>See abstract; and claim 1.                                                                                                                                                                                | 1-12                  |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

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

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

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

"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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

17 August 2016 (17.08.2016)

Date of mailing of the international search report

**18 August 2016 (18.08.2016)**

Name and mailing address of the ISA/KR

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**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/KR2015/013351**

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                               |                       |
|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

**PCT/KR2015/013351**

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