

Description

Title of Invention : Hollow Core-Shell Particles

Technical Field

The present invention generally relates to hollow core-shell particles having pores extending through its shell. The present invention also relates to a process for making these particles and for loading these particles with gas molecules. The present invention further relates to use of these loaded particles for the delivery of oxygen.

Background Art

During the event of respiratory failure or other circumstances where a patient stops breathing or when there is a lack of oxygenated blood to particular tissue or organs, doctors are allowed a very brief timeframe in which to take action before there is permanent damage in the body. This is caused by the accumulation of carbon dioxide and diminishing oxygen supply in the tissue, organs and/or whole body. If doctors or paramedics are unable to resume the supply of oxygenated blood, therapeutic options and survivability rapidly decrease. Therefore, it is presently a challenge, especially in emergency cases outside of a hospital setting, to minimize mortality, tissue necrosis or organ failure from such situations. Machines that artificially oxygenate blood and remove carbon dioxide are the only viable strategy; however they are marred by logistical limitations and are very costly.

In first aid, there is growing interest in wound dressings that can supply oxygen as this has been shown to speed up the healing process and afford better wound healing. Presently, doctors blow oxygen gas directly on the wound.

A growing trend in cosmetic skincare involves the use of oxygen in order to enhance the performance of skin regiments. Most skincare companies charge large premiums for skincare that oxygenates the skin, leading to improved appearance of skin.

Finally, researchers involved in tissue engineering, especially with stem cells, have found that there is a significant amount of necrosis when applying synthetic materials to repair or regenerate tissue. There is also a high number of incidents of stem cell death. Both of these are due to the lack of oxygen supply in such environments. Therefore, incorporating oxygen delivering particles or other means in such therapies would be desirable. A delayed or stimuli responsive release of oxygen would be highly desirable to improve the efficacy and success rate of such therapy.

The limitation presently faced is the poor solubility of O_2 in water. Techniques to supersaturate O_2 gas in water are still marred by thermodynamic and equipment limitations and therefore are still not viable options for providing O_2 rich solutions for applications that would require oxygenation.

Recently, nanosized hollow silica spheres were suggested as useful carriers of active species in solution for encapsulation and controlled delivery because of their biocompatibility, large surface area and internal void volume, adjustable void diameter and modifiable surface properties. However, these hollow core particles have not been used for

the delivery of gases. Hollow core particles would therefore be desirable that can encapsulate gas molecules and are functionalized in a way that they can take up and release gases upon a stimulus or in a delayed release pattern.

Therefore, there is a need to provide hollow core-shell particles that overcome, or at least ameliorate, one or more of the disadvantages described above.

There is also a need to provide hollow core-shell particles which provide a solution to the insolubility of gases in applications where gas delivery, especially oxygen delivery, is needed.

There is also a need to provide a process for preparing these hollow core-shell particles.

Summary

There is provided a hollow core-shell particle having pores extending through its shell and a plurality of polymer chains that are grafted to the outer surface of the shell, wherein the polymer backbone of said polymer chains comprises monomer units that are functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms.

According to a first aspect, there is provided a hollow core-shell particle having pores extending through its shell and a plurality of polymer chains that are grafted to the outer surface of the shell, wherein the polymer backbone of said polymer chains comprises monomer units that are functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms, wherein the particle is a hollow silica sphere

Advantageously, the polymer backbone may contain functionalities selected from either aminocarbonyls, amidines, guanidines, indoles or imidazoles. These nitrogen-containing functionalities may elicit a response to an external agent, for example to an increased concentration of carbon dioxide. This response may lead to the opening of the above mentioned core-shell particle.

Further advantageously, the hollow core may be filled with releasable gas molecules. These may be released upon the opening of the core-shell particle. They may also be released by diffusion from the particle core depending on the particle's environment.

Further advantageously, the releasable gas molecule may be oxygen.

According to a second aspect, there is provided a process for making a particle as disclosed above, comprising the steps of

- a) modifying the surface of a hollow particle with an amino, halogen, epoxide or thio-carbonyl thioester group;
- b) reacting the modified particle with a methacrylate monomer functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups; and

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- c) further polymerizing the obtained grafted hollow particle with the monomer of step b).

According to a third aspect, there is provided a process for making a particle as disclosed above, comprising the steps of

- a) modifying the surface of a hollow particle with an amino, halogen, epoxide or thio-carbonyl thioester group;

- b) reacting the modified particle with an optionally substituted acrylonitrile;
- c) further polymerizing the obtained grafted hollow particle with an acrylonitrile; and
- d) reacting the obtained cyano groups of the polymer backbone to amidine groups.

According to a fourth aspect, there is provided a process for loading the particle as disclosed above with a gas, comprising exposing the above mentioned polymer particle with a selected gas and allowing the gas to permeate into the hollow particle.

According to a fifth aspect, there is provided use of a loaded particle as disclosed above for the encapsulation and delivery of gases. Advantageously, the loaded particle may be used as a delivery means for oxygen in paramedic emergencies.

According to a sixth aspect, there is provided a delivery means comprising a plurality of particles as disclosed above, wherein the hollow core is filled with gas molecules. Advantageously, the delivery means functions as a carrier for the loaded particle and may deliver oxygen site-specific to oxygen-deprived tissues.

According to a seventh aspect, there is provided use of the delivery means as disclosed above to provide oxygen in cosmetic skin care applications.

According to an eighth aspect, there is provided a non-therapeutic use of the delivery means as disclosed above to deliver oxygen site-specific to an oxygen-deprived tissue or cell.

Definitions

The following words and terms used herein shall have the meaning indicated below:

The term 'hollow silica sphere' is to be interpreted broadly to include a hollow container which may consist of silica. The container contains a void. The void may refer to a partially enclosed space.

In the definitions of a number of substituents below it is stated that "the group may be a terminal group or a bridging group". This is intended to signify that the use of the term is intended to encompass the situation where the group is a linker between two other portions of the molecule as well as where it is a terminal moiety. Using the term alkyl as an example, some publications would use the term "alkylene" for a bridging group and hence in these other publications there is a distinction between the terms "alkyl" (terminal group) and "alkylene" (bridging group). In the present application no such distinction is made and most groups may be either a bridging group or a terminal group.

The term "aliphatic" is one that includes only carbon and hydrogen and possibly having monovalent and divalent straight or branched chain saturated or unsaturated hydrocarbon groups from 1 to 50 carbon atoms, preferably 1 to 30, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms or the likes. In cases where there is at least one double bond, either the E, Z, cis or trans stereochemistry may exist, where applicable, at any location along the carbon chain. An aliphatic group as used herein may be a terminal or bridging group.

The term "hetero-aliphatic" is one that includes "aliphatic" groups as mentioned above wherein the chains of the hydrocarbon groups may include hetero atoms such N, O or S.

The term "alicyclic" refers to a group that is both aliphatic and cyclic having from 3 to 30, preferably 6 to 30, carbon atoms and any other number of carbon atoms falling within this range. They contain one or more all-carbon rings which may be either saturated or unsaturated, but do not have aromatic character. Examples may include monocyclic cycloalkanes such as cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane,

cyclooctane, and so on. Bicyclic alkanes may include bicycloundecane and decalin. Polycyclic alkanes may include cubane, basketane, and housane. Such a group as used herein may be a terminal or bridging group.

The term "carbocyclyl" refers to a cyclic group of organic chemical compounds having from 3 to 12 ring atoms per ring, in which all the atoms comprising the ring are carbon atoms. The carbocycle may be saturated, partially saturated or unsaturated. The carbocycle may be monocyclic, multicyclic or fused. The group may be a terminal group or a bridging group.

The term "aromatic" as used herein refers to monovalent and divalent single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 30 carbon atoms. Examples of such groups include phenyl, biphenyl, naphthyl, phenanthrenyl, and the like. An aromatic group as used herein may be a terminal or bridging group.

The term "arylaliphatic" means an aryl-aliphatic-group in which the aryl and aliphatic moieties are as defined herein. Preferred arylalkyl groups contain a C_{1-30} alkyl moiety. Exemplary arylalkyl groups include benzyl, phenethyl, 1-naphthalenemethyl and 2-naphthalenemethyl. The group as used herein may refer to a terminal or bridging group.

The term "halogen" represents a chlorine, fluorine, bromine or iodine atom. Likewise, the term "halide" or "halo" represents a chloride, fluoride, bromide or iodide.

The term "heteratom" as used herein may refer to any suitable atom selected from N, O, S, Se and Si.

The term "alkylene" as used herein represents a substituent group or part of a group that refers to a straight or branched saturated aliphatic hydrocarbon group, preferably a C_1 - C_{12} alkylene, more preferably a C_1 - C_{10} alkylene, most preferably C_1 - C_6 or any of the number of carbon atoms falling within the stated range. Examples of suitable straight and branched C_1 - C_6 alkylene substituents include methyl, ethyl, n-propyl, 2-propyl, n-butyl, sec-butyl, t-butyl, hexyl, and the like. The group may be a terminal group or a bridging group.

The term "acyl" includes within its meaning an $R-C(=O)-$ group in which the R group may be an alkyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl group as defined herein. Examples of acyl include acetyl and benzoyl. The group may be a terminal group or a bridging group. If the group is a terminal group it is bonded to the remainder of the molecule through the carbonyl carbon.

The term "ester" includes within its meaning an $R-COO-$ group in which the R group may be a hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl group as defined herein. The group may be a terminal group or a bridging group. If the group is a terminal group it is bonded to the remainder of the molecule through the oxygen forming an ester linkage.

The term "amide" includes within its meaning an $R-CONR-R$ group in which the R group may be a hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl group as defined herein. The group may be a terminal group or a bridging group. If the group is a terminal group it is bonded to the remainder of the molecule through the carbon or nitrogen forming an amide linkage.

The term "carbonyl" includes within its meaning an R-C(=O)R group in which the R group may be a hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl or heteroaryl group as defined herein. The group may be a terminal group or a bridging group. If the group is a terminal group it is bonded to the remainder of the molecule through the carbon.

"Alkoxy" or "Alkyloxy" refers to an alkyl-O- group in which alkyl is as defined herein. These terms may be used interchangeably. Preferably the alkyloxy is a C₁-C₁₀alkyloxy. Examples include, but are not limited to, methoxy and ethoxy. The group as used herein refers to a terminal group.

"Alkenylene" as a group or part of a group denotes an aliphatic hydrocarbon group containing at least one carbon-carbon double bond and which may be straight or branched, preferably having 2-12 carbon atoms, more preferably 2-10 carbon atoms, most preferably 2-6 carbon atoms, in the normal chain. The group may contain a plurality of double bonds in the normal chain and the orientation about each is independently E, Z, *cis* or *trans* where applicable. Exemplary alkenylene groups include, but are not limited to, ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl and nonenyl. The group as used herein refers to a terminal group.

It is understood that alkenylene groups may be isomeric forms including diastereoisomers, enantiomers, tautomers, and geometrical isomers in "E" or "Z" configurational isomer or a mixture of E and Z isomers. It is also understood that some isomeric forms such as diastereomers, enantiomers, and geometrical isomers can be separated by physical and/or chemical methods and by those skilled in the art.

"Alkynylene" as a group or part of a group means an aliphatic hydrocarbon group containing a carbon-carbon triple bond and which may be straight or branched preferably having from 2-12 carbon atoms, more preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms in the normal chain. Exemplary structures include, but are not limited to, ethynyl and propynyl. The group as used herein refers to a terminal group.

"Cycloalkyl" refers to a saturated monocyclic or fused or spiro polycyclic, carbocycle preferably containing from 3 to 9 carbons per ring, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like, unless otherwise specified. It includes monocyclic systems such as cyclopropyl and cyclohexyl, bicyclic systems such as decalin, and polycyclic systems such as adamantane. A cycloalkyl group typically is a C₃-C₁₂ alkyl group. The group as used herein refers to a terminal group.

"Aryl" as a group or part of a group denotes (i) an optionally substituted monocyclic, or fused polycyclic, aromatic carbocycle (ring structure having ring atoms that are all carbon) preferably having from 5 to 12 atoms per ring. Examples of aryl groups include phenyl, naphthyl, and the like; (ii) an optionally substituted partially saturated bicyclic aromatic carbocyclic moiety in which a phenyl and a C₅₋₇ cycloalkyl or C₅₋₇ cycloalkenyl group are fused together to form a cyclic structure, such as tetrahydronaphthyl, indenyl or indanyl. The group as used herein refers to a terminal group. Typically an aryl group is a C₆-C₁₈ aryl group.

"Heterocycloalkyl" refers to a saturated monocyclic, bicyclic, or polycyclic ring containing at least one heteroatom selected from N, O, S, Se and Si, preferably from 1 to 3 heteroatoms in at least one ring. Each ring is preferably from 3 to 10 membered, more

preferably 4 to 7 membered. Examples of suitable heterocycloalkyl substituents include pyrrolidyl, tetrahydrofuryl, tetrahydrothiofuryl, piperidyl, piperazyl, tetrahydropyranyl, morpholino, 1,3-diazapane, 1,4-diazapane, 1,4-oxazepane, and 1,4-oxathiapane. A heterocycloalkyl group typically is a C₂-C₁₂ heterocycloalkyl group. The group as used herein refers to a terminal group.

"Heteroaryl" or "heteroaromatic heterocyclyl group" either alone or part of a group refers to groups containing an aromatic ring (preferably a 5 or 6 membered aromatic ring) optionally fused to another ring having one or more heteroatoms as ring atoms in the aromatic ring with the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include N, O, S, Se and Si. Examples of heteroaryl include thiophene, benzothiophene, benzofuran, benzimidazole, benzoxazole, benzothiazole, benzisothiazole, naphtho[2,3-b]thiophene, furan, isoindolizine, xantholene, phenoxatine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, tetrazole, indole including isoindole, 1H-indazole, purine, quinoline, isoquinoline, phthalazine, naphthyridine, quinoxaline, cinnoline, carbazole, phenanthridine, acridine, phenazine, thiazole, isothiazole, phenothiazine, oxazole, isooxazole, furazane, phenoxazine, 2-, 3- or 4- pyridyl, 2-, 3-, 4-, 5-, or 8- quinolyl, 1-, 3-, 4-, or 5- isoquinolyl, 1-, 2-, or 3- indolyl, and 2-, or 3- thienyl. A heteroaryl group is typically a C₁-C₁₈ heteroaryl group. The group as used herein refers to a terminal group.

"Non heteroaromatic heterocyclyl group" refers to a saturated or partly saturated monocyclic, bicyclic, or polycyclic ring containing at least one heteroatom selected from N, O, S, preferably from 1 to 3 heteroatoms in at least one ring.

"Amino acid derivative" either alone or part of a group refers to optionally substituted amino acids. The amino acids are comprising amine (-NH₂) and carboxylic acid (-COOH) functional groups. The definition includes the abundantly available 23 proteinogenic amino acids and their derivatives. As a group within a polymer or molecule the amino acid may be bound to the polymer chain or molecule via its amine group. The term includes non-substituted amino acids.

"Guanidine" either alone or part of a group refers to aNH₂-C(NH)-NH₂ or -NH-C(NH)-NH₂ group. As a group within a polymer or molecule the guanidine may be bound to the polymer chain or molecule via its amine group.

"Amidine" either alone or part of a group refers to a -C(NH)-NH₂ group. As a group within a polymer or molecule the guanidine may be bound to the polymer chain or molecule via its carbon atom.

"Thiocarbonyl thioester" either alone or part of a group refers to a -(S=)C-S- group.

A "bond" is a linkage between atoms in a compound or molecule. The bond may be a single bond, a double bond, or a triple bond.

The term "optionally substituted" as used herein means the group to which this term refers may be unsubstituted, or may be substituted with one or more groups independently selected from halogen, heteroatom, alkyl, acyl, ester, carbonyl, alkyloxy, alkenyl, alkynyl, sulfonamide, aminosulfonamide, cycloalkyl, aryl, heterocycloalkyl and a heteroaryl.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a hollow core-shell particle will now be disclosed.

There is provided a hollow core-shell particle having pores extending through its shell and a plurality of polymer chains that are grafted to the outer surface of the shell, wherein the polymer backbone of said polymer chains comprises monomer units that are functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms.

This invention provides hollow core-shell particles which have the function of encapsulating gas molecules in the hollow voids. The plurality of polymer chains, which may also be referred to as 'polymer brushes', may have the function of providing an environmentally dependent response to elicit release of the before mentioned encapsulated gas molecules. Suitable monomers of the polymer brushes may be selected for their ability to be responsive to external parameters, for example, to an increased concentration of carbon dioxide. Such monomers may contain nitrogen-rich functionalities, such as amidines and guanidines. They may contain amino acid derivatives such as tryptophan, arginine,

lysine, histidine and proline or their derivatives. They may also contain heteroaromatic or non heteroatomic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms. Optionally, the heterocycles may contain 1 to 2 nitrogen atoms, or 2 to 3 nitrogen atoms, or 1, 2 or 3 nitrogen atoms. Examples for these heterocycles may include aziridines, azirines, diazirines, oxaziridines, azetidines, azetes, diazetidines, pyrrolidines, pyrroles, imidazolidines, imidazoles, pyrazolidines, pyrazoles, oxazolidines, oxazoles, isoxazolidines, isoxazoles, thiazolidines, isothiazolidines, thiazoles, isothiazoles, triazoles, furazans, oxadiazoles, thiadiazoles, dithiazoles, piperidines, pyridines, piperazines, diazines, morpholins, oxazines, thiomorpholines, thiazines, triazines, azepanes, azepines, homopiperazines, diazepines, thiazepines, azocanes, azocines, indoles including isoindoles, quinolones, isoquinolines, benzazepines, carbazoles, acridines and dibenzoazepines.

As a consequence of using nitrogen-containing monomers, the polymer backbone, or polymer brush, may contain nitrogen-rich functionalities. Hence, in an optional embodiment, the polymer backbone may contain functionalities selected from either aminocarbonyls, amidines, guanidines, indoles or imidazoles. These nitrogen-containing functionalities may support in eliciting a response to an external trigger, for example to an increased concentration of carbon dioxide.

The polymer may be selected from, but not limited to, a polymer made from an amino acid derivative substituted acryl amide (such as methacrylamide). Alternatively or additionally, it may be made from a polymer of an amidine.

For example, the polymer may be selected from poly(histidine methacrylamide), poly(arginine methacrylamide) or poly(amidine).

At least one of the terminal ends of the polymer chains may be selected from a halogen moiety. The halogen moiety may be, for example, fluorine, chlorine, bromine or iodine. In one example, the halogen moiety is bromine.

The polymers are grafted to the outer surface of the shell. That means that they are covalently bonded to the particle. The particle can for instance be treated with a silane agent to introduce a binding group. The silane agent may be an amino group carrying silane. It may be 3-amino propyl triethoxy silane. The amino group may be further halogenated to form a halogen containing group for reaction with the polymer monomers. The linker in the grafting between the polymer and the particle may therefore be a silyl alkylene group, such as silyl propyl linker. The particle surface that carries amino functional or halogen functional groups may be contacted with the functionalized polymer monomer to bind the monomer to the particle surface, e.g. by reaction with a carboxy group or an unsaturated moiety of the monomer.

The hollow core-shell particle may be a hollow micro- or nanosphere, i.e. it includes a void or cavity. In typical embodiments, the microparticle or nanoparticle have a shell surrounding a void. The shell may be defined by a single wall with an internal and an external surface (i.e., balloon-like). The void or cavity may include gaseous molecules. The wall defining the shell has some pores, including for example holes, cracks and/or blowholes, which can be penetrated by gases. The particle may have any form and shape, including a sphere, a rod, a disc or a rope. The particle may preferably be a sphere. The

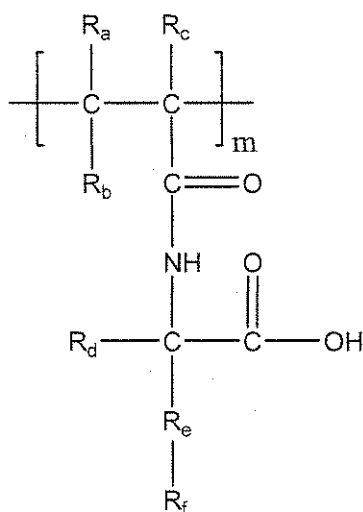
pores may be arranged in an ordered arrangement with symmetry such as hexagonal, cubic or lamellar.

The wall of the particle shell may be of small or even negligible thickness in comparison to the particle dimensions, such as less than about 10%, less than about 5%, less than about 2% or less than about 1% of the maximal particle width. The wall defining the shell may be porous (e.g. sponge-like) in nature. It may be microporous (diameters of less than 2 nm), mesoporous (diameters between 2 nm and 50 nm) or macroporous (diameters of greater than 50 nm).

The particle may have any surface area which is suitable for the present invention. For example, the particle may have a BET surface area in the range from about 10 m²/g to about 1000 m²/g, such as about 25 m²/g to about 500 m²/g, about 50 m²/g to about 250 m²/g, about 75 m²/g to about 200 m²/g or about 100 m²/g to about 300 m²/g, including a BET surface area of about 170 m²/g or about 160 m²/g. Other surface areas are possible.

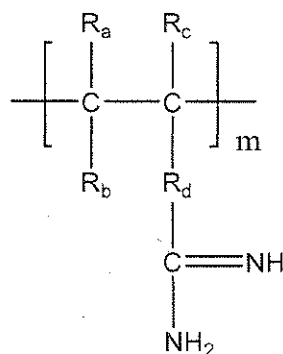
On the external surface area, i.e. the surface area on the exterior side of a shell surface of a particle of the invention, the polymer is grafted. The external surface area of shell-based particles may be measured by means of techniques known to those skilled in the art such as Atomic Force Microscopy (AFM) and BET isotherm analysis. The external surface area of particles of the invention may range from about 10 to about 500 square meters/gram. The internal surface area, i.e. the surface area facing the interior side of a shell surface of particles of the invention, is in contact with any matter that is included in the void of the particle, such as gas molecules. The internal surface area of shell-based particles with contiguous solid walls cannot be measured directly via techniques such as Atomic Force Microscopy (AFM) and BET isotherm analysis, but can be estimated based on the external particle surface area and particle wall thickness. The particle may be a hollow silica sphere. It may be a silica sphere containing a void. It may be a hollow sphere containing silica. The sphere may consist of silica. The void may refer to a 'container' or a partially enclosed space. In certain embodiments, it may be filled with gas molecules.

The polymer may consist of at least one repeating monomer unit of formula (Ia) or (Ib)



(Ia)

or



(Ib)

wherein

m is an integer selected in a way that the polymer has a molecular weight selected from the range of 2 kDa to 500 kDa;

R_a, R_b, and R_c represent a group that is independently selected from the group consisting of hydrogen, amine, optionally substituted aliphatic, optionally substituted hetero-aliphatic and optionally substituted carbocyclyl;

R_d represents hydrogen or a direct bond, or R_d represents optionally substituted C₁-C₆-alkyl or optionally substituted C₁-C₆-alkylidene.

R_e represents optionally substituted C₁-C₆-alkylidene;

R_f represents amino, optionally substituted heterocyclyl or an optionally substituted guanidinium group.

Optionally, R_a and R_b may represent hydrogen. Optionally, R_c may represent hydrogen, methyl or ethyl. Optionally, R_d may represent hydrogen or a direct bond. Optionally, R_e may represent C₁-C₆-alkylene. Optionally, R_f may represent an amino, aminocarbonyl, amidine, guanidine, indole or imidazole group.

The index m may be an integer from 1 to about 10000, such as 1 to about 9000, 1 to about 8000, 1 to about 7000, 1 to about 6000, 1 to about 5000, 1 to about 4000, 1 to about 3000 and 1 to about 2000. The index m may also be any integer not explicitly mentioned in the above listing. Typically the polymer defines a coating on the surface of the hollow silica particle. In one embodiment of the invention the polymer may be a substantially linear, i.e. substantially straight polymer. In some embodiments it may be hyperbranched polymer chains. The polymer will usually have a molecular weight in the range from about 500-2000000, such as about 500-1000000, 500-500000, 500-100000, 500-50000, 500-25000, 500-10000 or 500-5000. It should be noted that in the above formulas the order of the monomers is only exemplary, i.e. the order may be changed. Further, it should also be noted that the monomers may be present in different combinations, i.e. as alternating copolymers, periodic copolymers, statistical copolymers and/or block copolymers, wherein alternating copolymers are copolymers with regular alternating A and B units; periodic copolymers have A and B units arranged in a repeating sequence (e.g. (A-B-A-B-B-A-A-A-A-B-B-B)_n), statistical copolymers are copolymers in which the sequence of monomer residues follows a statistical rule, and block copolymers comprise two or more homopolymer subunits linked by covalent bonds. The union of the homopolymer subunits may require an intermediate non-repeating subunit, known as a junction block. Block copolymers with two or three distinct blocks are called diblock copolymers and triblock copolymers, respectively. In one embodiment three or more different copolymers may be used, wherein one of the monomers has to be functionalized with the above mentioned nitrogen atom containing groups.

The hydrodynamic diameter of the particle according to the invention may be between about 1 nm to 100 μm, preferably between 10 nm to 100 μm, or between about 10 nm to 90 μm, between about 10 nm to 80 μm, between about 10 nm to 70 μm, between about 10 nm to 60 μm, between about 10 nm to 50 μm, between about 10 nm to 40 μm, between about 10 nm to 30 μm, between about 10 nm to 20 μm, between about 10 nm to 10 μm, between

about 10 nm to 10 μ m, , between about 20 nm to 100 μ m, between about 30 nm to 100 μ m, between about 40 nm to 100 μ m, between about 50 nm to 100 μ m, between about 60 nm to 100 μ m, between about 70 nm to 100 μ m, between about 80 nm to 100 μ m, between about 90 nm to 100 μ m, between about 10 nm to 90 μ m, between about 20 nm to 80 μ m, between about 30 nm to 70 μ m, between about 40 nm to 60 μ m, or about 1 μ m, about 10 μ m, about 20 μ m, about 30 μ m, about 40 μ m, about 50 μ m, about 60 μ m, about 70 μ m, about 80 μ m, about 90 μ m, or about 100 μ m. It may be between about 350 to 750 nm, or between about 450 to 700 nm, or between about 550 to 750 nm, or between about 650 to 750 nm, or between about 350 to 650 nm, or between about 350 to 550 nm, or between about 350 to 450 nm, or between about 450 to 650 nm, or about 350 nm, 450 nm, 550 nm, 650 nm or 750 nm. The hydrodynamic diameter is calculated from measured data in a dynamic light scattering (DLS) experiment using the Stokes Einstein equation. By definition then, the DLS measured radius is the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination.

The diameter of the particle shell can be determined by TEM measurements. The diameter of the particle shell may be between about 1 nm to 100 μ m, or preferably 9 nm to about 90 μ m or between 10 nm and 100 μ m. It may be a smaller particle of between about 9 nm to 9 μ m, or between about 300 to 700 nm, or between about 400 to 700 nm, or between about 500 to 700 nm, or between about 600 to 700 nm, or between about 300 to 600 nm, or between about 300 to 500 nm, or between about 300 to 400 nm, or between about 400 to 600 nm, or about 300 nm, 400 nm, 500 nm, 600 nm or 700 nm.

The particle may contain about 10 % to 90 % (w/w) of the polymer, or about 20 % to 90 % (w/w) of the polymer, or about 30 % to 90 % (w/w) of the polymer, or about 40 % to 90 % (w/w) of the polymer, or about 50 % to 90 % (w/w) of the polymer, or about 60 % to 90 % (w/w) of the polymer, or about 70 % to 90 % (w/w) of the polymer, or about 80 % to 90 % (w/w) of the polymer, or about 10 % to 80 % (w/w) of the polymer, or about 10 % to 70 % (w/w) of the polymer, or about 10 % to 60 % (w/w) of the polymer, or about 10 % to 50 % (w/w) of the polymer, or about 10 % to 40 % (w/w) of the polymer, or about 10 % to 30 % (w/w) of the polymer, or about 10 % to 20 % (w/w) of the polymer, or about 20 % to 80 % (w/w) of the polymer, or about 30 % to 70 % (w/w) of the polymer, or about 40 % to 60 % (w/w) of the polymer, or about 10 %, or about 20 % (w/w) of the polymer, or about 30 % (w/w) of the polymer, or about 40 % (w/w) of the polymer, or about 50 % (w/w) of the polymer, or about 60 % (w/w) of the polymer, or about 70 % (w/w) of the polymer, or about 80 % (w/w) of the polymer, or about 90 % (w/w) of the polymer. Typically the surface has a polymer content/polymer density of at least about 20% (w/w), including at least about 25% (w/w), at least about 30% (w/w), at least about 35% (w/w), at least about 40% (w/w), or at least about 60% (w/w), or at least 70 % (w/w) or at least 80 % (w/w). The polymer content can be determined by thermogravimetric analysis (TGA) by heating the particles up to 800 °C at a rate of 10 °C/min.

The before mentioned hollow-core shell particle may have a shell which is microporous , mesoporous or macroporous. Microporous matter is in the art understood to have pores of a width of less than about 2 nm, whereas mesoporous matter is understood as having pores of about 2 nm to about 50 nm. The particle may for instance have a porous shell with a pore width of about 0.1 nm to about 500 nm, including a pore width of about 0.1 nm to about 8 nm, of about 0.5 nm to about 6 nm, of about 1 nm to about 6 nm, of about 1 nm to about 10

nm, about 1 nm to about 50 nm, about 1 nm to about 100 nm, about 1 nm to about 250 nm, about 1 nm to about 500 nm, such as a pore width of about 1 nm. A respective mesoporous or hollow shell core particle may have any form and shape, including a sphere, a rod, a disc or a rope. The pores in the shell may be unordered or arranged in an ordered arrangement with symmetry such as hexagonal, cubic or lamellar. Additionally or alternatively, the pores may function as 'molecular sieves'. They may have the ability to selectively sort molecules based primarily on a size exclusion process. This may be due to a very regular pore structure. The maximum size of the molecular or ionic species that can enter the pores of a particle may be controlled by the diameter of the channels.

The pores on the particle according to the invention may have an average diameter between about 0.1 Å to 50 nm, or between about 0.5 Å nm to 50 nm, or between about 1 Å to 50 nm, or between about 0.1 nm to 50 nm, or between about 0.3 nm to 30 nm, or between about 0.4 nm to 50 nm, or between about 0.01 nm to 40 nm, or between about 0.01 nm to 20 nm, or between about 1 nm to 20 nm, or between about 0.01 nm to 10 nm, or between about 0.1 nm to 20 nm, or between about 0.1 nm to 30 nm, or between about 0.1 nm to 25 nm, or about 0.01 nm, or about 5 nm, or about 0.02 nm, or about 0.2 nm, or about 2 nm, or about 20 nm, or about 50 nm.

The pores and the hollow core of the particle can be analysed by a variety of techniques. Examples include, but are not limited to, transmission electron microscopy, scanning electron microscopy, gas, e.g. nitrogen, adsorption, inverse platinum replica imaging, small-angle X-ray scattering, and positron annihilation lifetime spectroscopy. In some embodiments of transmission electron microscopy (TEM) a series of TEM images is taken from the same position at different tilt angles and 3D-information obtained in the so called tomography mode. In some embodiments of scanning electron microscopy (SEM) high resolution-SEM is used, working at very low currents and voltages. Structural information can furthermore be taken from NMR, Raman and FTIR spectroscopies, electrochemical methods, UV-Vis absorption and fluorescence spectroscopies, as well as single molecule spectroscopic methods. In single molecule spectroscopic methods the materials are typically investigated by doping them with very low, usually nanomolar concentrations of fluorescent dyes. Individual molecules and/or individual nanoscale environments can then be analysed.

The hollow core may be filled with releasable gas molecules. Alternatively, it may be in a vacuum state and not contain any, or close to none, releasable gas molecules. The gas molecules may consist of 2 to 4 atoms. The gas molecules may be selected from the group consisting of oxygen, nitrogen, carbon dioxide, nitrous oxide and carbon monoxide. In the examples, the gas is selected from oxygen and nitrogen.

Preferably, the gas is oxygen.

The particle may show an interaction upon exposure to an agent in the environment of the particle or upon temperature changes. Hence, the nanoparticle may be unloaded by an external trigger depending on the medium it is in. It may be loaded depending on the medium it is in. In other words, the particle may have an 'on-off' switch for loading or unloading the gas. It may also be described as an 'open and closed position' mechanism, whereas the open position may refer to the unloading mechanism and the closed position may refer to the loaded particle containing the encapsulated gas. Illustrative in this regard

may be Fig 1. Fig. 1 is a schematic drawing of a core-shell particle encapsulating and releasing compounds in response to an environmental trigger. In the example chosen, the external trigger is represented by nitrogen and carbon dioxide.

The interaction may be a change in particle size or pore size. For example, the specific binding of gases may affect the hydrodynamic radius. Illustrative in this context may be Fig. 2. Fig. 2 is a schematic drawing of the effect of an external agent to the hydrodynamic radius. The hydrodynamic radius in the 'closed' position, i.e. without the triggering external agent, may be the smallest, while 'opening up' it increased and the particles are drifting further apart. In the 'open' position, the pores of the core-shell particle are open and the loaded gas may depart from the core-shell particle.

The above mentioned interaction may lead to the release of the gas from the hollow core. This may result in an 'open position' as mentioned above.

The agent in the environment may be carbon dioxide. Hence, a carbon dioxide rich environment may be a trigger for releasing the gas which is being encapsulated in the particle. It may be the switch for changing from the loaded into the unloaded position.

In a second aspect, there is provided a process for making a particle of any of the preceding claims, comprising the steps of

- a) modifying the surface of a hollow particle with an amino, halogen, epoxide or thio-carbonyl thioester group;
- b) reacting the modified particle with a methacrylate monomer functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups; and
- c) further polymerizing the obtained grafted hollow particle with the monomer of step b).

The starting materials of the process can be hollow core-shell particles prepared according to methods known in the art. The hollow core-shell particle may be a hollow silica sphere. For example, a silica sphere or other hollow particles can be produced as substantially disclosed in US 20130034609 A1 or the references cited therein. They may be produced, in a first step, by reaction of a polyvinyl moiety with a polymer monomer in a polar solvent and a polymerisation initiator. This may produce a first polymer colloid. In one example, a polystyrene colloid is produced from polyvinyl pyrrolidone with styrene and 2,2'-azobis(2-methylpropionamide) dihydrochloride in deionized water. Further reaction with a silicate in a basic environment followed by drying and calcination may then produce the hollow silica sphere. In one example, tetraethylorthosilicate is used as the silicate.

Alternatively hollow silica can be synthesized according to a previously reported method (Zhu, J., et al. (2010). "Ultrasmall, Well-Dispersed, Hollow Siliceous Spheres with Enhanced Endocytosis Properties." *Small* 6(2): 276-282). In step a) of the above mentioned process, the hollow core-shell particle may be functionalized with an amino, halogen, epoxide or thio-carbonyl thioester group. It is preferably modified with amino or halogen groups. This can be achieved by reaction of the surface of the particle with a reagent that can be bound to the surface and carries directly or after further modification an amino,

halogen, epoxide or thio-carbonyl thioester group. In case of using a silica particle the reagent may be an amino silane. In one embodiment, 3-amino propyl triethoxy silane is used in this reaction.

In case an amino functional group has been introduced to the particle surface, it is possible to react such functionalized particle with a halogenating agent in order to introduce a halogen group by replacing the amino group. Any common halogenating agent may be used. Examples of halogenating agents may be, but are not limited to, N-bromosuccinimide, 2-bromoisobutryl bromide and so on.

An epoxide group may be introduced in step a). In case of using a silica particle the reagent may be an epoxy modified silane. In one embodiment, 3-glycidyloxypropyl)trimethoxy silane is used in this reaction. An epoxide modified particle is then obtained.

In case an epoxide functional group has been introduced to the particle surface, it is possible to react such functionalized particle with a with a RAFT agent in order to introduce a thio-carbonyl thioester group. Any common RAFT agent (e.g. those commercially available from Sigma-Aldrich or Boron Molecular) may be used. Examples of acid-RAFT agents may be, but are not limited to, 3-(((1-carboxyethyl)thio)carbonothioyl)thio) propanoic acid, 4-(((2-carboxyethyl)thio)carbonothioyl)thio)-4-cyanopentanoic acid, 4-cyano-4-(((dodecylthio) carbonothioyl)thio) pentanoic acid, 2-(phenylcarbonothioylthio)propanoic acid, 3-(benzylsulfanyl thiocarbonyl sulfanyl)-propionic acid, 4-cyanopentanoic acid dithiobenzoate and so on. This reaction may be also performed in inert organic solvents such as xylene or toluene at elevated temperatures optionally under inert gas. A base, such as an amine base, may be added.

The process of step a) may be carried at an elevated temperature of about 50° C to about 120° C . In some embodiments, it may be carried out under an inert gas atmosphere.

The solvents in step a) are preferably inert organic solvents such as xylene, toluene for the silane reaction , or methylene chloride or chloroform for the halogenation.

The principle modification reaction is for instance described in US 20130034609 A1.

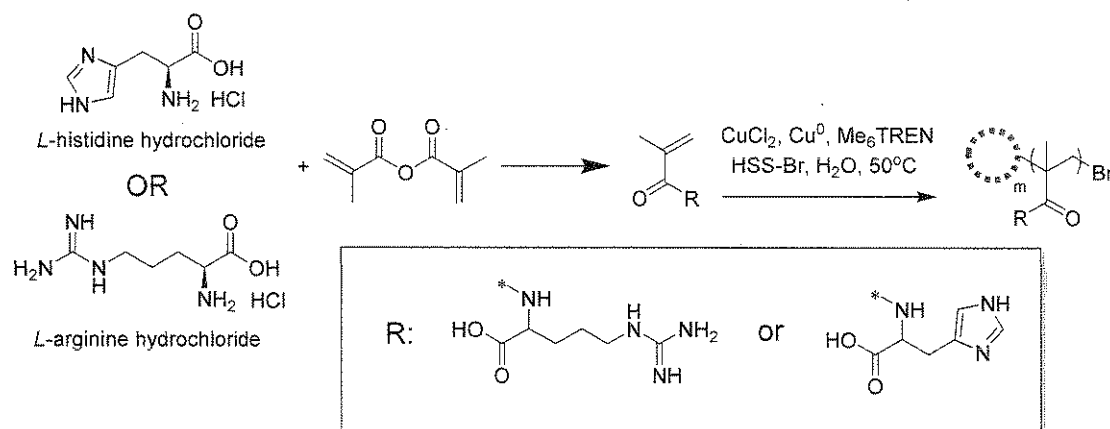
The starting materials of step b) comprise reaction products of acrylates with amino acids or their derivatives. The amino acids me be selected from tryptophan, lysine, arginine, histidine and proline. The amino acid may be reacted in the form of its hydrochloride with an acrylic anhydride, such as methacrylic anhydride under reduced temperature, such as about 3 to 10 °C in an aqueous alkaline solution as substantially described in the example.

The starting materials may be other monomers, such as methacrylates, that comprise a heteroaromatic or non heteroaromatic heterocyclic group, wherein the heterocycles comprise 1 to 3 nitrogen atoms. Those starting materials are commercially available or can be easily prepared by methods known in the art.

In step b) of the process, the modified particle may further be reacted with a starting material to form the grafted polymer chains under polymerisation conditions known to the person skilled in the art (see for instance US 20130034609 A1).

The process of coupling the monomer and the further polymerisation (step c) to the particle surface may in some embodiments be carried out under an inert gas atmosphere. The process may be carried out at room temperature or at an elevated temperature. The temperature may for example be selected in the range from about 30° C to about 120° C, about 40° C to about 100° C or about 50° C to about 100° C, e.g. about 50° C. The process may also be carried out under an inert gas atmosphere, i.e. in the presence of gases that are not reactive, or at least not reactive to a detectable extent, with regard to the reagents and solvents used. Examples of a reactive inert atmosphere are nitrogen or a noble gas such as argon or helium. The process may take up to several days, such as about 10 hours, about 24 hours, about 48 hours, about 60 hours or about 72 hours. The monomers may contain acrylate functionality. An acrylate may be grafted to a halogen functionalized particle by a SET LRP reaction. The SET LRP can be started using an amine, such as tris[2-(dimethylamino)ethyl]amine (Me_6TREN), in the presence of Cu(II) salts and Cu^0 in an aqueous solution. The acrylic monomer is then grafted on the modified hollow particle (step b) and subsequently polymerized (step c). Step b) and step c) may be carried out in a 'one-pot' sequence under the same conditions in a suitable inert organic solvent. By carrying out this process, a core-shell particle having polymer chains of the structure of (Ia) may be obtained.

In one embodiment, the synthetic scheme for the synthesis of the core-shell particle as disclosed may be as follows:



In this scheme, the circle signifies the hollow silica sphere (HSS), which may be modified with a bromine functionality (HSS-Br).

Step b) and c) may further involve a radical polymerisation utilizing the introduced epoxide or thio-carbonyl thioester group for grafting the polymer to the particle core.

In this embodiment of step b) of the process, the modified particle may further be reacted with a starting material to form the grafted polymer chains under RAFT polymerisation conditions generally known to the person skilled in the art (e.g. Sun, JT, Yu, ZQ, Hong, CY, Pan, CY, *Macromol. Rapid Commun.*, 2012, 33, 811-818).

The process of coupling the monomer and the further polymerisation (step c) to the particle surface may then in these embodiments be carried out under an inert gas atmosphere. The process may be carried out at room temperature or at an elevated temperature. The temperature may for example be selected in the range from about 30° C to about 100° C,

about 40° C to about 70° C or about 45° C to about 65° C, e.g. about 60° C. The process may also be carried out under an inert gas atmosphere, i.e. in the presence of gases that are not reactive, or at least not reactive to a detectable extent, with regard to the reagents and solvents used. Examples of a reactive inert atmosphere are nitrogen or a noble gas such as argon or helium. The process may take up to several days, such as about 10 hours, about 24 hours, about 48 hours, about 60 hours or about 72 hours. The monomers may contain acrylate functionality. An acrylate may be grafted to a thio-carbonyl thioester functionalized particle by a Reversible Addition-Fragmentation chain Transfer (RAFT) reaction. The RAFT reaction can be started using a known free radical initiator, such as 4,4'-azobis(4-cyanovaleric acid). The acrylic monomer is then grafted on the modified hollow particle (step b) and subsequently polymerized (step c). Step b) and step c) may be carried out in a 'one-pot' sequence under the same conditions in a suitable inert organic solvent. By carrying out this process, a core-shell particle having polymer chains of the structure of (Ia) may be obtained.

In a third aspect, there is provided a process for making a particle comprising the steps of

- a) modifying the surface of a hollow particle with an amino or halogen group;
- b) reacting the modified particle with an optionally substituted acrylonitrile;
- c) further polymerizing the obtained grafted hollow particle with the monomer of step b); and
- d) reacting the obtained cyano groups of the polymer backbone to amidine groups.

In this aspect, step a) may be identical to step a) of the second aspect.

The starting material in step b) besides the reaction product of step a) is an optionally substituted acrylonitrile. Acrylonitrile is a commercially available polymer monomer. Optionally substituted acrylonitriles are also commercially available or can be made according to methods known in the art. In step b), the modified particle is grafted with an acrylonitrile or its derivative, which is subsequently polymerized under conditions known to the person skilled in the art (step c).

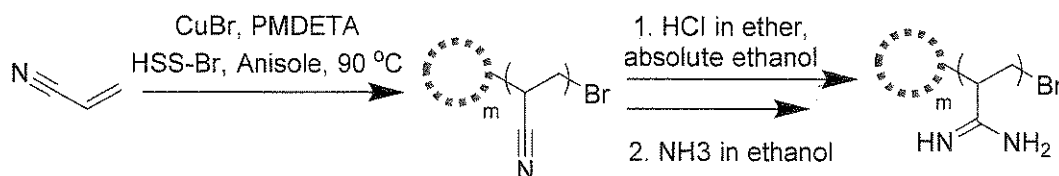
The process of coupling the acrylonitrile and the further polymerisation (step c) to the particle surface may in some embodiments be carried out under an inert gas atmosphere. The process may be carried out at room temperature or at an elevated temperature. The temperature may for example be selected in the range from about 60° C to about 120° C, about 80° C to about 100° C, e.g. about 90° C. The process may also be carried out under an inert gas atmosphere, i.e. in the presence of gases that are not reactive, or at least not reactive to a detectable extent, with regard to the reagents and solvents used. Examples of a reactive inert atmosphere are nitrogen or a noble gas such as argon or helium. The process may take up to several days, such as about 10 hours, about 24 hours, about 48 hours, about 60 hours or about 72 hours. Acrylonitrile may be grafted to a halogen functionalized particle by running an ATRP reaction. The ATRP reaction can be started using a Cu^I salt, in an inert organic solvent and the presence of an amine, such as N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA).

After the polymerisation step c) which can be run in a one pot with step b), the nitrile functionality may be reacted to an amidine functionality.

The process of reacting a cyano group to an amidine group may in some embodiments be carried out under an inert gas atmosphere. The process may be carried out at room temperature or at an elevated temperature. The temperature may for example be selected in the range from about 20° C to about 30° C. In a first step the product of step c) is reacted with a strong mineral acid, such as HCl for about 5 to 24 hours in the presence of an inert solvent, such as ether. In a second step the conversion product is obtained after reaction by using ammonia in a polar solvent such as ethanol.

Following this process, a core-shell particle having a polymer chain of structure (Ib) may be obtained.

In one embodiment, the synthetic scheme for the synthesis of the core-shell particle as disclosed may be as follows:



In this scheme, the circle signifies the hollow silica sphere (HSS), which may be modified with a bromine functionality (HSS-Br).

In a fourth aspect, there is provided a process for loading the particle as disclosed above with a gas, comprising exposing the above mentioned polymer particle with a selected gas as disclosed above and allowing the gas to permeate into the hollow particle.

This process may include placing the particles into a container commonly used for protection gas techniques, such as a Schlenk flask, and degassing the container under vacuum for about 1 to 10 hours, or about 2 to 10 hours, or about 3 to 10 hours, or about 4 to 10 hours, or about 5 to 10 hours, or about 6 to 10 hours, or about 7 to 10 hours, or about 8 to 10 hours, or about 9 to 10 hours, or about 1 to 9 hours, or about 1 to 8 hours, or about 1 to 7 hours, or about 1 to 6 hours, or about 1 to 5 hours, or about 1 to 4 hours, or about 1 to 3 hours, or about 1 to 2 hours, or about 1 hour, about 2 hours, or about 3 hours, or about 4 hours, or about 5 hours, or about 6 hours, or about 7 hours, or about 8 hours, or about 9 hours, or about 10 hours. Afterwards the container may be backfilled with the gas the particle is to be loaded with, for example oxygen, for about 1 to 10 hours, or about 2 to 10 hours, or about 3 to 10 hours, or about 4 to 10 hours, or about 5 to 10 hours, or about 6 to 10 hours, or about 7 to 10 hours, or about 8 to 10 hours, or about 9 to 10 hours, or about 1 to 9 hours, or about 1 to 8 hours, or about 1 to 7 hours, or about 1 to 6 hours, or about 1 to 5 hours, or about 1 to 4 hours, or about 1 to 3 hours, or about 1 to 2 hours, or about 1 hour, about 2 hours, or about 3 hours, or about 4 hours, or about 5 hours, or about 6 hours, or about 7 hours, or about 8 hours, or about 9 hours, or about 10 hours. The particles are kept in a sealed container until use.

Separately, a solution suitable for medication delivery or cosmetics delivery, such as a phosphate buffered saline solution, may be prepared. The loaded particles can then be

immersed into the solution and used in the desired application immediately. A kit of parts comprising the gas loaded particles of the invention in a sealed container together with another container with a suitable solution for the application, such as a buffer solution (e.g. a PBS solution) can be mentioned as an embodiment of the invention. The sealed container has an extended shelf life. The hollow particle of the invention may in some embodiments be water-soluble. Where desired, such a water-soluble microparticle or nanoparticle may be designed for sustained and for controlled delivery of gases, especially oxygen. In a fifth aspect, there is therefore provided use of a loaded particle as disclosed above for the encapsulation and delivery of gases.

In a sixth aspect, there is provided a delivery means comprising a plurality of particles as disclosed above, wherein the hollow core is filled with gas molecules. This delivery means may be any carrier or solution being capable of delivering the loaded particles to the site in need of the gas. It may be a saline solution for direct injection, or a solution of dextrose in water. It may be a topical cream. In one example, it is the before mentioned phosphate buffered saline solution.

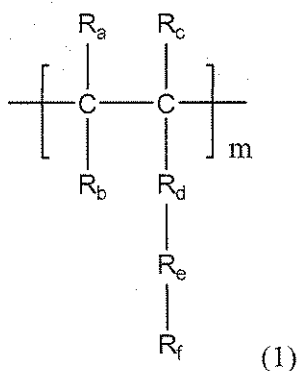
The delivery means may provide oxygen in paramedic or skin care applications. Advantageously, the delivery means may act as a transporter or a carrier for oxygen in emergency situations. In a sustained system oxygen is delivered over a prolonged period of time by the particles in a dispersion, which overcomes the problems of making oxygen available as gas solution in liquids. The term 'controlled' indicates that control is exerted over the way in which oxygen is delivered to the tissues once it has been administered to the organism to be treated, e.g. the patient.

The delivery means may deliver oxygen site-specific to an oxygen-deprived tissue. This may be used in paramedic emergencies, wherein there is insufficient time to transport the patient to a hospital. This delivery means may be used immediately at the emergency site. Illustrative in this aspect may be Fig. 3. Fig. 3 is a schematic drawing of a core-shell particle, containing the silica sphere loaded with oxygen gas and polymer brushes which, upon being triggered by a deoxygenated red blood cell (RBC), opens and degrades, thereby releasing oxygen to re-oxygenate the RBC.

In the present invention, gas filled hollow core-shell particles as delivery means are obtained. The shells provide the stability, whereas the polymer brushes can trigger the carbon dioxide responsive properties. Even very low concentrations of carbon dioxide may be sufficient.

Further statements relating to the invention are now being disclosed:

1. A core-shell particle having pores extending through its shell and a plurality of polymers that are bonded to the outer surface of the shell, wherein said polymers are comprised of repeating monomer units of formula (1):



wherein

m is at least 1;

R_a , R_b , R_c and R_e are independently selected from the group consisting of a hydrogen, amine, optionally substituted aliphatic, optionally substituted hetero-aliphatic and optionally substituted carbocyclyl;

R_d is selected from the group consisting of a bond, optionally substituted hetero-aliphatic, optionally substituted aliphatic group, optionally substituted alkoxy, carbonyl, imine optionally substituted imidamide, optionally substituted amide and optionally substituted carbocyclyl;

R_f is absent when R_e is a heteroatom but is present when R_e is an optionally substituted aliphatic, optionally substituted hetero-aliphatic and optionally substituted carbocyclyl such that R_f is selected from the group consisting of optionally substituted aliphatic, optionally substituted hetero-aliphatic, optionally substituted carbocyclyl, optionally substituted hetero-aryl, optionally substituted hetero-aliphatic guanidium groups, as well as other substituted basic amino acids including arginine, histidine and lysine.

2. The core-shell particle according to statement 1, wherein said optionally substituted aliphatic is selected from the group consisting of optionally substituted C_{1-30} alkylene, optionally substituted C_{2-30} alkenylene and optionally substituted C_{2-30} alkynylene.

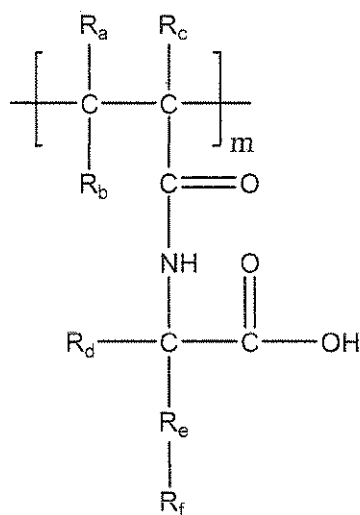
3. The core-shell particle according to statement 1 or 2, wherein said optionally substituted hetero-aliphatic contains one or more heteroatoms selected from N, O, S, Se or Si, and is selected from the group consisting of optionally substituted C_{1-30} -heteroalkylene, optionally substituted C_{2-30} -heteroalkenylene and optionally substituted C_{2-30} -heteroalkynylene.

4. The core-shell particle according to any one of the preceding statements, wherein said optionally substituted carbocyclyl is selected from the group consisting of optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl and optionally substituted heterocycloalkyl.

5. The core-shell particle according to any one of the preceding statements, wherein said R_a , R_b , R_c and R_e are independently a hydrogen or an optionally substituted aliphatic.

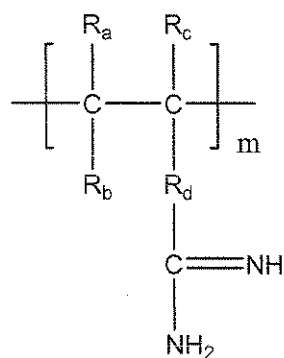
6. The core-shell particle according to any one of the preceding statements, wherein said R_a , R_b and R_e are hydrogen.

7. The core-shell particle according to any one of the preceding statements, wherein said R_c is a C_{1-30} alkyl.
8. The core-shell particle according to any one of the preceding statements, wherein said R_d is an optionally substituted ester having the formula $-R_g'CO_2R_g$, an optionally substituted amide having the formula $-R_g'CONHR_g$, an optionally substituted imidamide having the formula $-R_g'CNHNHR_g$, an optionally substituted imine having the formula $-R_g'CNHNR_g$ wherein R_g' and R_g is each independently selected from the group consisting of an C_{1-30} alkylene, C_{2-30} alkenylene, C_{2-30} alkynylene, C_{1-30} -heteroalkylene, C_{2-30} -heteroalkenylene and C_{2-30} -heteroalkynylene.
9. The core-shell particle according to statement 8, wherein said R_g is C_2 -alkylene and R_g' is C_3 -alkenylene.
10. The core-shell particle according to any one of the preceding statements, wherein said formula (1) is formula (1a) or formula (1b):



(1a)

or



(1b)

wherein R_a , R_b , R_c , R_d , R_e and m are as defined in any one of the preceding statements;

R_f is selected from the group consisting of hydrogen, optionally substituted aliphatic, optionally substituted hetero-aliphatic and optionally substituted carbocyclyl.

11. The core-shell particle of statement 10, wherein said polymer of formula (1a) is either poly(histidine methacrylamide) or poly(arginine methacrylamide).
12. The core-shell particle of statement 10, wherein said polymer of formula (1b) is poly(amidine).
13. The core-shell particle according to any one of the preceding statements, wherein said polymer has a molecular weight selected from the range of 2 kDa to 500 kDa.

14. The core-shell particle according to any one of the preceding statements, wherein said polymer is coupled to the particle via a bridging group selected from the group consisting of an aminoalkyl silane, haloalkyl silane and an aminoalkoxysilane.
15. The core-shell particle according to any one of the preceding statements, wherein said particle contains 10 % to 90 % (w/w) of the polymer of formula (1).
16. The core-shell particle according to statement 21, wherein said particle contains 20% to 80% (w/w) of the polymer of formula (1).
17. The core-shell particle according to any one of the preceding statements, wherein said particle is a microparticle or a nanoparticle with a hydrodynamic diameter of 1 nm to 100 μm .
18. The core-shell particle according to statement 24, wherein the hydrodynamic diameter is in the range of 10 nm to about 10 μm .
19. The core-shell particle according to any one of the preceding statements, wherein the pores of said particle are microporous, mesoporous or macroporous.
20. The core-shell particle according to any one of the preceding statements, wherein the pores on the particle have an average diameter between 1 nm to 50 nm.
21. The core-shell particle according to any one of the preceding statements, wherein said core-shell particle further comprises an inner void.
22. The core-shell particle according to any one of the preceding statements, wherein said pores on said particle alternate between an "open" position and a "closed" position in response to an external stimulus.
23. The core-shell particle according to statement 22, wherein said external stimulus is dissolved carbon dioxide concentration.
24. The core-shell particle according to statement 22 or 30, wherein in said "open" position, said core-shell particle allows the exchange of an agent between the core of said particle and an external environment.
25. The core-shell particle according to statement 22 or 24, wherein in said "closed" position, said core-shell particles does not allow the exchange of an agent between the core of said particle and an external environment.
26. The core-shell particle according to any one of statements 22 to 25, wherein when said carbon dioxide is present, said pores are in said "open" position and when said carbon dioxide is substantially absent, said pores are in said "closed" position.
27. The core-shell particle of statement 26, wherein when said polymer is poly(arginine methacrylamide), poly(histidine methacrylamide) or poly(amidine), said polymer is responsive to carbon dioxide.
28. The core-shell particle of any one of statements 21 to 27, wherein said agent is a fluid or dispersed in a fluid.

29. The core-shell particle of any one of the preceding statements, wherein said particle comprises silica.
30. A method of synthesizing a core-shell particle according to any one of statements 1 to 29, comprising the steps of:
- i) providing a polymeric grafted core-shell particle; and
 - ii) reacting said polymeric grafted core-shell particle with a monomer of formula (1) to form said core-shell particle.
31. The method of statement 30, wherein said polymeric grafted core-shell particle comprises a polymer selected from a halo-poly(alkane).
32. A method of transferring an agent into or out of the inner void of the core-shell particle according to any one of statements 1 to 29, comprising the step of altering the temperature or carbon dioxide concentration of a solution that is in contact with said core-shell particle.
33. A method of loading a gas into the inner void of the core-shell particle according to any one of statements 1 to 29, comprising the step of exposing said core-shell particle to a gaseous environment containing said gas and allowing said gas to permeate into said inner void.
34. A delivery agent comprising a plurality of core-shell particles according to any one of statements 1 to 29, wherein the core of said particles contains an agent.
35. The delivery agent according to statement 33, wherein said agent is a chemical compound, molecule or a gas.
36. A pharmaceutical composition comprising a plurality of core-shell particles according to any one of statements 1 to 29, wherein the core of said particles contains an agent.
37. The pharmaceutical composition according to claim 35, wherein said agent is a drug, protein, gene, or oxygen.

Brief Description of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig.1

[Fig. 1] is a schematic drawing of a core-shell particle encapsulating and releasing compounds in response to an environmental trigger.

Fig.2

[Fig. 2] is a schematic drawing of the effect of an external agent to the hydrodynamic radius.

[Fig. 2a] shows the aggregated particles with polymers in a hydrophobic 'closed' position.

[Fig. 2b] shows the dispersed particles with polymers gaining hydrophilicity but still in a 'closed' position.

[Fig. 2c] shows the dispersed particles with polymers in a fully extended 'open' position.

Fig.3

[Fig. 3] is a schematic drawing of a core-shell particle, containing the silica sphere loaded with oxygen gas and polymer brushes which upon being triggered by a deoxygenated red blood cell (RBC), opens and degrades, thereby releasing oxygen to re-oxygenate the RBC.

Fig.4

[Fig. 4a] shows a TEM image of the unmodified HSS

[Fig. 4b] shows a TEM image of the polymer grafted HSS

Fig.5

[Fig. 5] shows the TGA of pristine HSS, HSS-g-P(Amd), HSS-g-P(HistM) and HSS-g-P(ArgM). The TGA results show a polymer content of approximately 17%, 29% and 70% respectively.

Fig.6

[Fig. 6] shows the measured dissolved oxygen (DO) content profiles as a function of time for a degassed PBS solution injected with the various oxygen loaded nanoparticles (5 mg/mL) at $t=3$. All data is normalized to starting dissolved oxygen and weight % of silica.

Fig.7

[Fig. 7] shows the measured dissolved oxygen (DO) content profiles as a function of time for a degassed PBS solution injected with the various oxygen loaded nanoparticles (5 mg/mL) at $t=3$ mins normalized to starting dissolved oxygen, weight % of silica and the total DO content released over a span of 30 minutes (integral of curves from Fig. 6). Solid lines indicate data fitted to a first order exponential decay function where $y=Ae^{-\lambda t}$.

Fig.8

[Fig. 8] shows the relative cell viability in the L929 cell line after exposure to the various HSS-g-polymer (nanoparticles) and positive control polymer, PEI (0.03125 – 1 mg/mL). Relative cell viability was determined using the MTT Assay and expressed as a percentage of untreated cells (control).

Examples

Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Materials

Inhibitors were removed from all monomers by passing it over a short layer of basic alumina. All solid monomers were dissolved in THF, then rapidly passed over a short layer of basic alumina to remove the inhibitor. Copper bromide was purified by refluxing in glacial acetic acid for 18 hours, washing it with ether then drying it extensively under vacuum. N,N,N',N',N''- Pentamethyl diethylenetriamine (PMDETA) was distilled before use and copper strips were etched with 1M sulfuric acid before use. For cytotoxicity studies, L929 mouse fibroblast cells were purchased from American Type Culture Collection (ATCC, Rockville, MD). Cells are maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mg penicillin, 100 µg/mL streptomycin at standard conditions, 37 °C, 5 % CO₂ and 95 % relative humidity. 1- (4,5-dimethyl-thiazol-2-yl)-3,5-diphenylfor-mazan (MTT) (Sigma Aldrich) was used as received. MTT solution (5mg/mL) was prepared in phosphate buffered saline (PBS; pH 7.4), protected from light and filtered before use. All other chemicals were used as received.

Example 1

Experiment (a): Synthesis of 500 nm hollow silica spheres (HSS):

0.5 µm sized hollow silica spheres were synthesized by firstly dissolving 0.5 g of polyvinyl pyrrolidone ($M_n = 40,000 \text{ g.mol}^{-1}$) in deionized water (100 mL) with stirring at room temperature for 4 hours. To this, styrene (11 g) and 2,2'-azobis(2-methylpropionamide) dihydrochloride (0.26 g) were added and degassed with argon for 30 minutes, then allowed to react for 24 hours at 70 °C. The mixture was cooled and 18 mL of the mixture was extracted and mixed with ethanol (240 mL) and ammonia (25 wt%, 12mL). Separately, tetraethylorthosilicate (3.2 mL) was mixed with ethanol (5 mL) and added dropwise to the mixture of polystyrene colloid, ethanol and ammonia at 50 °C. This mixture was then allowed to react for 24 hours. The solution was centrifuged to concentrate the suspended particles, washed three times with ethanol, dried, and calcined at 550 °C. The particles were characterized by BET and visualized by TEM.

Alternative synthesis of silica spheres (for reference):

Pluronic F108 (1.0 g) and mesitylene (1.0g) were added to HCl (2.0M, 30mL) and stirred vigorously for 6 h. Then, tetraethyl orthosilicate (1.0g) was added dropwise to the surfactant solution under vigorous stirring and the mixture was allowed to react for 12h followed by the addition of dimethoxy dimethyl silane (0.5 g). The solution is allowed to react for an addition 48h. The milky product mixture was dialyzed for 48h against water with a semi-permeable membrane with a molecular weight cutoff of 12-14 kDa. The

dialyzed solution was freeze dried to yield a white powder which was calcined at 550 °C for 6 h.

Example 2

Experiment (b): Bromide functionalization of HSS (HSS-Br) for ATRP:

The hollow silica spheres (0.2 g) obtained in part (a) were dispersed in p-xylene (10 mL), followed by the addition of 3-amino propyl triethoxy silane (0.3 mL). The mixture was allowed to stir for 24 hours at 90 °C under argon, then washed several times with diethyl ether, and then filtered. The collected solids were dried in a vacuum oven, then redispersed in a mixture of anhydrous chloroform (40 mL) and triethylamine (1.2 mL). This flask was immersed in ice while 2-bromoisobutyryl bromide (0.6 mL) in anhydrous chloroform (4 mL) was added dropwise over a period of 1 hour, after which the reaction mixture was removed from ice and allowed to react at ambient temperature for a further 18 hours. At the end of the reaction, the solids were filtered and washed several times with chloroform, then dried under vacuum.

Alternative functionalization with epoxide to HSS-RAFT particles:

The epoxide functionalized HSS nanoparticles were made by first mixing 0.2 g of HSS into 10 mL of p-xylene and dispersed in solution by sonication for 30 min. The solution was then heated to 90 °C under argon and 0.3 mL of (3-glycidyloxypropyl)trimethoxy silane was added. The mixture was allowed to react for 24 h. The mixture was cooled to room temperature, filtered and washed thoroughly with diethyl ether. The HSS-epoxide nanoparticles were dried under vacuum then redispersed with an equal mass of (3-(benzylsulfanylthiocarbonylsulfanyl)-propionic acid) (0.2 g) in 10 mL of toluene. Triethyl amine (107 μ L, 0.77 mmol) was then added and the mixture was allowed to react for 24 hours at room temperature. The product was separated by filtration and washed with acetone to yield a pale yellow powder. The particles were analyzed by thermogravimetric analysis (TGA) for their weight percent content (14%). The sulfur content of the product, found by elemental analysis is 1.49%.

Example 3

Experiment (c): Synthesis of HSS-graft-poly(amidine) (HSS-graft-P(Amd)) via ATRP:

A monomer solution was first prepared by adding acrylonitrile (5 g, 94.2 mmol) to anhydrous DMF (15 mL) in a sealed flask. This solution was purged with nitrogen gas for 1 hour. The bromide functionalized HSS (0.2 g) were first dispersed and sonicated in anhydrous anisole (5 mL) in a schlenk flask. To this, 0.02 g of CuBr was added, the flask sealed and purged with nitrogen gas for 30 minutes. PMDETA (40 μ L) was then added to the schlenk flask using a gas tight syringe, and the solution is purged with nitrogen for a further 30 minutes. At the end of this time period, the monomer solution was transferred to the schlenk flask via a cannula, and the solution was reacted at 90 °C for 9 hours. The product was then centrifuged and washed with acetone to remove the solvent, copper and unreacted monomer. To further remove any residual copper, the solids were redispersed in acetone and mixed with Dowex Marathon MSC (H^+) ion exchange resin. The resin was

removed, solids were dried and then redispersed in absolute ethanol (15 mL), sealed in a flask and chilled to 0 °C. To this, HCl (15 mL, 1 M) in ether was added dropwise and the mixture was thereafter allowed to warm up to room temperature and left to react overnight. The product was washed and centrifuged three times with absolute ethanol, then redispersed in absolute ethanol (15 mL). To this, ammonia (10 mL, 2 M) in ethanol was added and the solution was reacted at room temperature for 4 hours, after which the product is washed and centrifuged three times with ethanol. The washed solids were dried under vacuum to yield the nanoparticles. The particles were analyzed for the weight percent of polymer by TGA.

Alternative polymerisation with HSS-RAFT:

HSS-RAFT nanoparticles (0.1g) were dispersed in 10 mL of toluene in a Schlenk flask by sonication. 2 g of the methacrylamide or acrylonitrile monomer and 4,4'-azobis(4-cyanovaleric acid) (3.4 mg, 0.012 mmol) were then added and the Schlenk flask was sealed. The mixture was subjected to 3 freeze-pump-thaw cycles to remove all dissolved gasses and the reaction was left at 60 °C for 18 hours. To terminate the polymerization, the Schlenk flask was unsealed and compressed air was blown into it. The particles were then separated by centrifugation, re-suspended and centrifuged 3 times in acetone to remove all unreacted monomer, initiator and ungrafted polymer.

Example 4

Experiment (d): Synthesis of histidine methacrylamide (HistM) and arginine methacrylamide (ArgM):

Histidine or arginine methacrylamide monomers were prepared similarly. Arginine hydrochloride (20.0 g, 0.0950 mol) or histidine hydrochloride (19.9 g, 0.0950 mol) and sodium bicarbonate (16.8 g, 0.2 mol) was added to 100 mL of water. The solution was cooled to 5 °C, and methacrylic anhydride (12.5 mL, 0.11 mol) was added dropwise to the solution. The solution was reacted for 30 mins, following which aqueous ammonia was added to raise the pH value of the solution to 8. Unreacted solids were filtered and the filtrate was washed three times with dichloromethane. The aqueous layer was collected, freeze dried, then stored at -20 °C.

Example 5

Experiment (e) Synthesis of HSS-graft-P(HistM) and HSS-graft-P(ArgM) via SET-LRP:

Both silica grafted polymers were prepared similarly. A monomer solution was first prepared by adding arginine methacrylamide (6.22 g, 25.5 mmol), or histidine methacrylamide (6 g, 25.5 mmol) to 12.44 mL or 12 mL of deionized water respectively. The solution was then purged with nitrogen gas for 1 hour. The bromide functionalized HSS (0.2 g) were first dispersed and sonicated in acetone (2 mL) in a schlenk flask. To this, CuCl₂ (0.0017 g) and a 1x1 mm strip of copper tape was added. The flask was sealed and purged with nitrogen gas for 30 minutes. Me₆TREN (35 µL) was then added via a gas tight syringe. The suspension was allowed to purge with nitrogen for a further 30 minutes. The monomer solution was transferred via a cannula to the schlenk flask, and the mixture was reacted at 50 °C for 18 hours. At the end of the reaction, the copper tape was removed, and

the product was centrifuged and washed with water to remove remnant copper and monomer. The solids were then redispersed in 5 mL of deionized water, and then freeze dried. The particles were analyzed for the weight percent of polymer by TGA.

Example 6

Experiment (f): Preparation of oxygen loaded nanoparticle solutions:

The nanoparticles were placed in a sealed schlenk flask and placed under vacuum for 2 hours to ensure full evacuation of air from the nanoparticles. The schlenk flask was then backfilled with oxygen for 2 hours to fill the evacuated spaces with oxygen gas. Separately, a solution of phosphate buffered saline (PBS) was purged with nitrogen gas to displace all other dissolved gasses. This solution was then added to the oxygenated nanoparticles (5 mg/mL) with a gas tight syringe. The mixture was agitated briefly to fully disperse the nanoparticles.

Example 7

Experiment (g): Testing of the effects of the nanoparticle solution to the oxygen saturation of degassed PBS:

Dissolved oxygen release studies were performed on a Rank Brothers Digital Model 10 Dissolved Oxygen (DO) meter that was calibrated with oxygen saturated PBS solution. The dissolved oxygen content was measured for the degassed PBS solution prepared in part (f) and was found to be 30.5 %. To this solution 1 mL of the nanoparticle solution from part (f) was added and dissolved oxygen percentages were recorded for a period of 4 hours.

Example 8

Experiment (h): Cell Viability Testing with MTT:

Cytotoxicity of the nanoparticles was assessed on L929 mouse fibroblast cells. L929 cells were seeded in 96-well cell culture plates (1×10^5 cells/mL) and incubated under standard conditions for 24 hr. Cell culture medium was then replaced with medium containing serial dilutions of the nanoparticle or control polymer polyethylenimine (PEI), (0.03125 – 1 mg/mL) and incubated for 4 hr. The culture medium was then removed and replaced with fresh DMEM and incubated for 42 hrs. 10 μ L of filtered MTT (5mg/mL) was added and cells were incubated for an additional 4 hr. Unreacted dye was removed by aspiration and formazan crystals were dissolved with 100 μ L/well DMSO. The absorbance was measured at 570 nm (SpectraMax Plus384, Molecular Devices). 6 replicate measurements were performed and the relative cell viability is expressed as:
$$\frac{[A_{570}(\text{polymer} + \text{cells}) - A_{570}(\text{blank})]}{[A_{570}(\text{cells only}) - A_{570}(\text{blank})]} \times 100 \%$$

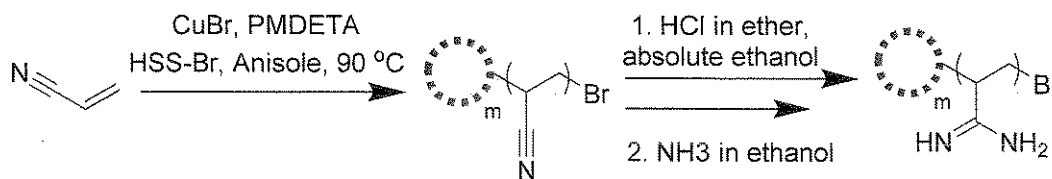
Results

Half micron sized hollow silica particles were targeted in the examples of the invention. To synthesize the particles, a solution of polystyrene latex was first prepared. The suspended polystyrene particles act as a template for a sol-gel deposition of silica via the Stöber process, which yields a mesoporous silica shell. Subsequently, the particles were calcined in a furnace at 550 °C to burn off the polystyrene template. The particles were visualized by

TEM and an image of the particle formed is shown in Fig. 4a. The particle size is approximately 0.5 μm , with a 50 nm silica shell. BET analysis revealed that the particles have a surface area of 230 m^2/g and an average pore size of 45 Å.

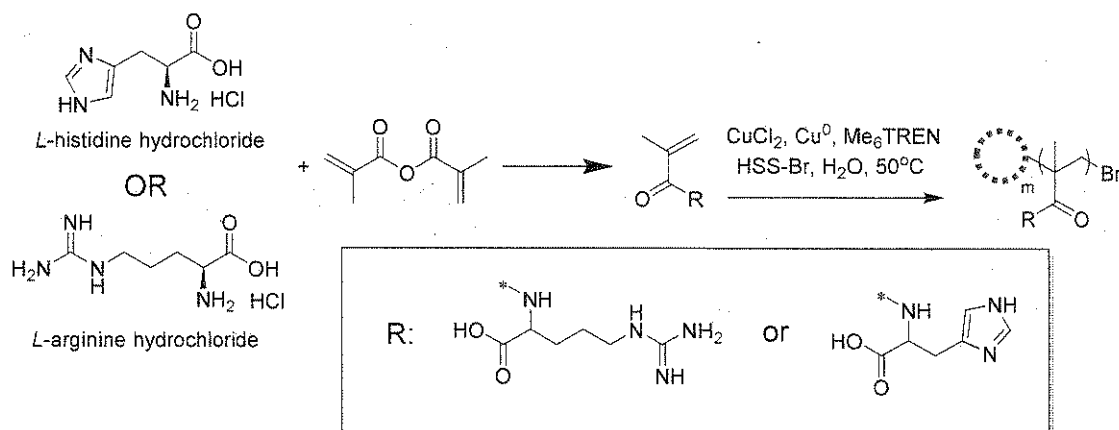
In order to graft polymers to the surface, the hollow silica surface were modified with a bromide functionality. The bromide functionality serves as an atom transfer radical polymerization (ATRP) initiating moiety. This was done by a sequential surface modification of hydroxyl groups to primary amine groups by reacting with 3-aminopropyl triethoxysilane, then by modifying primary amine groups to bromide groups by reacting with 2-bromoisobutyryl bromide. Once the silica particles were modified with bromide groups, surface initiated ATRP or single electron transfer-living radical polymerization (SET LRP) was performed.

The polymerization of acrylonitrile on the HSS surface was performed in anisole in the presence of copper bromide and PMDETA over 8 hours at 90 °C. At the end of the polymerization, the particles were first washed thoroughly with acetone then dried under vacuum. They were placed in a round bottom flask, redispersed in absolute ethanol and reacted with hydrogen chloride for 18 hours, then washed thoroughly in ethanol. The product was then further reacted with ammonia in absolute ethanol to yield the final HSS-g-P(Amd). The overall reaction scheme for this polymerization was shown in Scheme 1. This silica grafted polymer was visualized by TEM (Fig 4b) and analyzed for polymer content by TGA (17 %).



Scheme 1: The synthetic pathway to generate HSS-graft-P(Amd)

The arginine and histidine methacrylamide monomers were synthesized by reacting the amino acids with methacrylic anhydride to yield white powders. These monomers were polymerized on the HSS surface in deionized water in the presence of copper (II) chloride, copper wire, Me₆TREN, at 50 °C for 18 hours. At the end of the polymerization, the remnant copper wire was removed, then the particles were washed thoroughly with deionized water and freeze dried. The overall reaction schemes for these polymerizations are shown in Scheme 2. The polymer content of the product was determined by TGA and was found to be 29% and 70% for HSS-g-P(HistM) and HSS-g-P(ArgM) respectively. The TGA curves for all polymer-silica materials are shown in Fig. 5.



Scheme 2: The synthetic pathway to generate HSS-graft-P(ArgM) or HSS-graft-P(HistM)

The particle size in water and phosphate buffered saline (PBS) was characterized by DLS. A 1 mg/mL solution of the nanoparticles was prepared in the respective solvents. The solutions were either sparged with nitrogen or carbon dioxide gas and the resultant particle sizes, measured over 10 readings, are reported in Table 1.

Table 1: Particle size measurements for all the HSS particles in both deionized water and PBS when sparged with either nitrogen or carbon dioxide gas.

	In deionized water		In PBS	
	With N ₂	With CO ₂	With N ₂	With CO ₂
HSS	932 ± 23 nm	893 ± 22 nm	985 ± 24 nm	1008 ± 25 nm
HSS-g-P(Amd)	3230 ± 360 nm	3660 ± 230 nm	4130 ± 250 nm	5790 ± 453 nm
HSS-g-P(HistM)	8260 ± 360 nm	3620 ± 160 nm	4850 ± 260 nm	4040 ± 100 nm
HSS-g-P(ArgM)	2230 ± 60 nm	3380 ± 130 nm	3590 ± 220 nm	5290 ± 370 nm

There was a significant change in hydrodynamic radius with respect to the presence of carbon dioxide in both deionized water and PBS. In both cases, HSS-g-P(HistM) shrank, while HSS-g-P(Amd) and HSS-g-P(ArgM) expanded when the sample was bubbled with CO₂. Conversely, plain HSS particles had approximately the same particle size, regardless of solution and dissolved gas, which, as expected suggested that there is no effect of the solution on the particle size of silica. It is noteworthy that the sizes of all the polymer grafted particles in a carbonated solution were approximately the same, and therefore, it is likely that the polymer chains on the particle surface were similarly solvated in the carbonated state. Hence, such a result would suggest that in the non-carbonated state, the particles either shrink, as in the case of HSS-g-P(Amd) and HSS-g-P(ArgM), or aggregate, as in the case of HSS-g-P(HistM). All polymer grafted particles have a larger hydrodynamic radius in PBS, and it can be attributed to ionic and electrostatic interactions with the buffer.

In order to demonstrate the efficacy of the nanoparticles in oxygenating solutions, an oxygen release study was performed. There were three main objectives for the oxygen

release study: 1) to determine the increase in dissolved oxygen upon the dosage of the particles; 2) to determine the release profile, whereby the change in dissolved oxygen content of the solution was charted with respect to time; 3) the total oxygen delivered over the 30 minute period.

To prepare oxygen loaded nanoparticles, the particles were weighed out into schlenk flasks, the flasks were sealed, and then placed under vacuum for 2 hours to remove all air. After the two hours, the schlenk flask was backfilled with oxygen gas at 1 atmosphere and left to equilibrate for 2 hours. Separately, PBS was sparged with nitrogen gas for 1 hour to remove dissolved gases. The degassed PBS was then transferred to the schlenk flask to make up a 5 mg/mL solution of oxygen filled nanoparticles. In the oxygen release experiments, nitrogen sparged solution of PBS was first placed in an oxygen sensor to measure the baseline oxygen content of degassed PBS. The solution was allowed to equilibrate for 3 minutes after which an equal volume of the oxygen-filled nanoparticle solution was added to the PBS. Since the samples were exposed and uncovered, it is likely that there was gas exchange taking place with the atmosphere. The dissolved oxygen content was measured every minute over 30 minutes. Fig. 6 shows the change in dissolved oxygen over time for the different nanoparticle solutions (5 mg/mL) added at $t=3$ mins, normalized to silica content.

An instantaneous spike to peak DO concentration was observed at $t=4$ mins for HSS, HSS-g-P(Amd) and HSS-g-P(Arg) systems as encapsulated oxygen was released and the gas was dissolved into the degassed PBS reservoir. Following this, the DO levels began to decay immediately. The reason for this decay is due to equilibration of the sample with atmospheric gases and the consumption of oxygen by the DO probe. A similar DO spike and decay curve was observed for both HSS and HSS-g-P(Amd), while the shape of the HSS-g-P(ArgM) is similar but shows a significantly higher elevation of DO content. In the case of HSS-g-P(HistM), peak DO concentration occurs much later at $t=8$ mins, 5 minutes post injection. This gradual release is indicative of different release mechanism and diffusion kinetics of oxygen as compared to the other materials.

In addition to the shape of the curves, it is also important to analyze the amount of oxygen that can be delivered by each of the different nanoparticle systems. It is particularly important to understand the total oxygen delivered with respect to the mass of silica as a basis of comparison for each nanoparticle system. These quantities were determined by the integral from $t=0$ -30 mins of each of the DO curves in Fig. 6, and are reported in Table 2.

Table 2: Integrals of each dissolved oxygen curves.

Material	Integral
HSS	476.85
HSS-g-P(Amd)	534.34
HSS-g-P(HistM)	563.52
HSS-g-P(ArgM)	2800.05

The disparity in the DO content delivered by each of the nanoparticles suggests that there could be several factors governing the loading and delivery of oxygen via this route. Firstly, it is clear from all release profiles that there is gas encapsulation and release within the hollow silica. All polymer grafted systems show a higher total amount of DO per weight of

silica. This highlights the potential of using polymer grafted particles to modulate gas diffusion kinetics and encapsulation efficacy. Moreover, there is a direct correlation with the amount of polymer grafted per particle (weight percent) versus the amount of DO delivered. The HSS-g-P(ArgM) with the highest polymer content (weight percent), delivers the highest amount of DO over 30 mins with respect to silica weight as compared to HSS which has no polymer. This could suggest that the size or amount of grafted polymer brushes play a vital role in the encapsulation of oxygen, whereby they entrap oxygen gas within the polymeric matrix and provide a more secure encapsulation of oxygen due to a thick polymer brush, which would inherently slow down the transport of oxygen from the nanoparticles.

The DO release curves from Fig. 6 were normalized to the integral value from Table 2 and reported in Fig. 7 to provide a comparison between the decay kinetics of each release curve. The normalized data sets were fit to a first order exponential decay function with the form $y=Ae^{-\lambda t}$ where A is a constant, t is the time in minutes and λ is the decay rate. The value of λ captures the rate of diffusion, equilibration of DO with atmospheric gases and consumption of oxygen by the oxygen probe. As the equilibration of DO with atmospheric gases and consumption of oxygen by the probe are assumed to be the same for all systems the λ values are meaningful values for the purposes of comparison. The values of the decay rates for the respective systems are listed in Table 3. The release curve of HSS shows a high spike in DO content, which decays the most rapidly. HSS-g-P(Amd) and HSS-g-P(HistM) show a similar decay profile, but with a 1.9 (94 %) and 2.6 times (157 %) slower decay in oxygen content respectively compared to bare HSS. These results further demonstrate that the grafted polymer brushes are crucial for the prolonged or stimuli responsive release of oxygen and their delivery kinetics are modulated by the polymer type. HSS-g-P(Amd) still shows a 30% decrease in the decay rate as compared to HSS, however the fitted kinetic parameter suggest a more rapid release/less sustained delivery of oxygen compared to the HSS-g-P(Amd) and HSS-g-P(HistM) materials.

Table 3: Decay rates for each of the normalized DO curves in Fig. 7.

Material	Decay Rate (λ)	Error
HSS	0.095	± 0.002
HSS-g-P(Amd)	0.073	± 0.003
HSS-g-P(HistM)	0.049	± 0.007
HSS-g-P(ArgM)	0.037	± 0.004

The DO decay profile, along with the DLS data and area under the curve reveal that each particle has a unique mechanism for oxygen release, which is dependent on the type of polymer brushes as well as the amount of polymer grafted on the particle surface. Ungrafted HSS could prove useful for applications where an instant oxygen release is required. HSS-g-P(Amd) shows a fairly rapid release of O_2 as well, which could also be presumably due to the low polymer content, while still having a response to CO_2 . This suggests that if a stimuli dependent response is preferred over the non-stimuli responsive HSS, such a grafted polymer could be considered. HSS-g-P(ArgM) delivers much more oxygen and shows a slower diffusion kinetics to HSS-g-P(Amd), which would suggest that if more O_2 is required for a longer period this type of grafting would be preferable. HSS-g-P(HistM) shows a sustained and delayed release with peak O_2 concentrations only

occurring 5 minutes post injection. If a delayed and sustained release is preferred, this would be the ideal choice.

Finally, basic toxicity studies are performed for the nanoparticles via a MTT assay on L929 mouse fibroblast cells (Fig. 8). PEI, a standard positive control polymer commonly used in gene transfection studies was also tested for comparative purposes. For all nanoparticle solutions, 80-100 % of viable cells were detected for the entire concentration range under study. This is in stark contrast to PEI, where even at exposure to the lowest concentration results in less than 20 % cell viability. Of the three nanoparticle solutions, HSS-g-P(HistM) exhibits the highest relative toxicity, at slightly above 80 %. This however, should not be of significant concern as histidine based polymers have been earmarked and heavily tested as they have been identified as biocompatible polymers (E.B. Anderson, T.E. Long, Polymer, 2010, 2447-2454). The MTT assay shows that all of the synthesized materials are virtually non-toxic to fibroblast cells. This can be attributed to the bioinspired design of the grafted polymers, taking derivatives and intermediates from naturally occurring motifs found in biology, in this case-amino acids.

The low toxicity, coupled with a stimuli response to CO₂ which allows for the delivery of oxygen gas makes the synthesized particles a highly viable option for both topical and in vivo oxygen delivery as well as artificial respiration.

Industrial Applicability

This invention provides hollow particles that can be used to capture gas molecules and deliver these gas molecules upon triggering the gas release. The particles can be easily loaded with those gas molecules. The polymers grafted on the particle shells allow to trigger a release when the particle is at the location of delivery. The new particles made by the process according to the invention can be used in numerous applications where the delivery of gases is complicated, but the particles can be transported to by a carrier.

In one embodiment this invention seeks to supply oxygen in response to the presence of carbon dioxide via oxygen encapsulated polymer-silica nanoparticles. The polymers may be derived from either methacrylate functionalized amino acids (L-arginine & L-histidine), or from polyacrylonitrile that has been converted to hold amidine functionality. The polymers serve as “gatekeepers” on the porous hollow silica particles by mediating their hydrodynamic radius in response to the presence of carbon dioxide. Molecules such as oxygen gas can be encapsulated within the hollow silica and released when required. The oxygen filled hollow silica particles can be injected into a patient’s bloodstream and can deliver oxygen intravenously, especially when the particles are exposed to dissolved carbon dioxide. This serves as a nano-sized breathing apparatus that oxygenates a patient’s blood while providing clinicians a larger timeframe in which to take action. Therefore, incorporating these particles in such therapies would provide a delayed or stimuli responsive release of oxygen, which would be highly desirable to improve the efficacy and success rate of such therapy.

The polymers that can be used in the invention are non-toxic and show almost no cell toxicity even at high concentrations. The polymers are bioinspired and are synthesized from either common amino acids (histidine and arginine), or derived from common industrial

polymers such as acrylonitrile (to form polyamidine), thereby keeping potential production costs low.

The purpose of such a supply can be extended to various medical applications ranging from emergency medicine to basic first aid. These materials can also be used in skincare formulations.

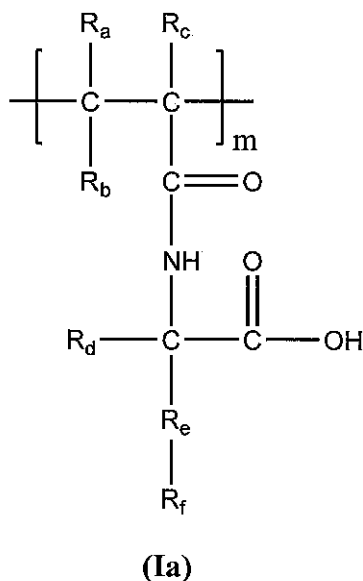
Due to low cost and toxicity, these particles are viable not only for biomedical applications but can also extend their use to consumer care applications.

However, this concept is not limited to the encapsulation of oxygen gas. The present technology should be extended to encapsulate other gaseous compounds that require release or modulation of diffusion kinetics in solutions. Gases typically have low solubility in water and other solutions. Using such nanoparticle systems may be able to temporarily increase the incorporated gas in solutions. This concept can also be extended towards active molecules such as drugs as the potential applications of this technology into drug delivery are vast.

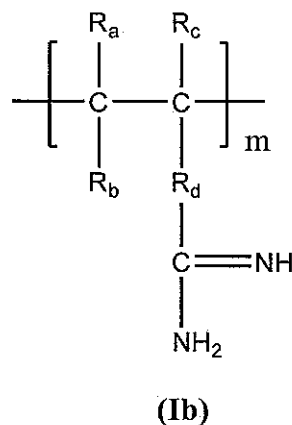
It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A hollow core-shell particle having pores extending through its shell and a plurality of polymer chains that are grafted to the outer surface of the shell, wherein the polymer backbone of said polymer chains comprises monomer units that are functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms, wherein the particle is a hollow silica sphere.
2. The particle of claim 1, wherein the polymer backbone comprises functionalities selected from either aminocarbonyls, amidines, guanidines, indoles or imidazoles.
3. The particle of claim 1, wherein the polymer is selected from poly(histidine methacrylamide), poly(arginine methacrylamide) or poly(amidine).
4. The particle of any of the preceding claims wherein the terminal end of the polymer chains comprises a halogen moiety.
5. The particle of any of claim 1 or 2, wherein the polymer comprises at least one repeating monomer unit of formula (Ia) or (Ib)



or



wherein

m is an integer selected in an way that the polymer has a molecular weight selected from the range of 2 kDa to 500 kDa;

R_a, R_b, and R_c represent a group that is independently selected from the group consisting of hydrogen, amine, optionally substituted aliphatic, optionally substituted hetero-aliphatic and optionally substituted carbocyclyl;

R_d represents hydrogen or a direct bond, or R_d represents optionally substituted C₁-C₆-alkyl or optionally substituted C₁-C₆-alkylidene.

R_e represents optionally substituted C₁-C₆-alkylidene;

R_f represents amino, optionally substituted heterocyclyl or an optionally substituted guanidinium group.

6. The particle of claim 5, wherein

R_a, and R_b represent hydrogen;

R_c represents hydrogen, methyl or ethyl;

R_d represents hydrogen or a direct bond;

R_e represents C₁-C₆-alkylene;

R_f represents an amino, aminocarbonyl, amidine, guanidine, indole or imidazole group.

7. The particle of any of the preceding claims, wherein the hydrodynamic diameter of the particle is between about 10 nm to 100 μ m.

8. The particle of any of the preceding claims, wherein the diameter of the particle shell is between about 10 nm to 100 μ m.

9. The particle of any of the preceding claims, wherein said particle contains 10 % to 90 % (w/w) of the polymer.

10. The particle of any one of the preceding claims, wherein said hollow-core shell particle has a shell that is microporous, mesoporous or macroporous.

11. The particle according to any one of the preceding claims, wherein the pores on the particle shell have an average diameter between 0.1 Å to 50 nm.

12. The particle according to any one of the preceding claims, wherein the hollow core is filled with releasable gas molecules.

13. The particle of claim 12 wherein the gas is oxygen.

14. The particle according to any of the preceding claims which shows an interaction upon exposure to an agent in the environment of the particle or upon temperature changes.

15. The particle according to claim 14, wherein the interaction is a change in particle size or pore size.

16. The particle according to claim 15 wherein the interaction leads to the release of the gas from the hollow core.

17. The particle of any of claims 14 to 16 wherein the agent in the environment is carbon dioxide.

18. A process for making a particle of any of the preceding claims, comprising the steps of
- a) modifying the surface of a hollow particle with an amino, halogen, epoxide or thio-carbonyl thioester group;
 - b) reacting the modified particle with a methacrylate monomer functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups; and
 - c) further polymerizing the obtained grafted hollow particle with the monomer of step b).
19. A process for making a particle of any of claims 1 to 17, comprising the steps of
- a) modifying the surface of a hollow particle with an amino, halogen, epoxide or thio-carbonyl thioester group;
 - b) reacting the modified particle with an optionally substituted acrylonitrile;
 - c) further polymerizing the obtained grafted hollow particle with an acrylonitrile; and
 - d) reacting the obtained cyano groups of the polymer backbone to amidine groups.
20. A process for loading the particle of any of claims 1 to 17 with a gas, comprising exposing said polymer particle with said gas and allowing the gas to permeate into the hollow particle.
21. Use of a loaded particle prepared by the process of claim 20 for the encapsulation and delivery of gases.
22. A delivery means comprising a plurality of particles according to any of claims 1 to 17, wherein the hollow core is filled with gas molecules.
23. Use of the delivery means of claim 22 to provide oxygen in cosmetic skin care applications.
24. A non-therapeutic use of the delivery means of claim 22 to deliver oxygen site-specific to an oxygen-deprived tissue or cell.

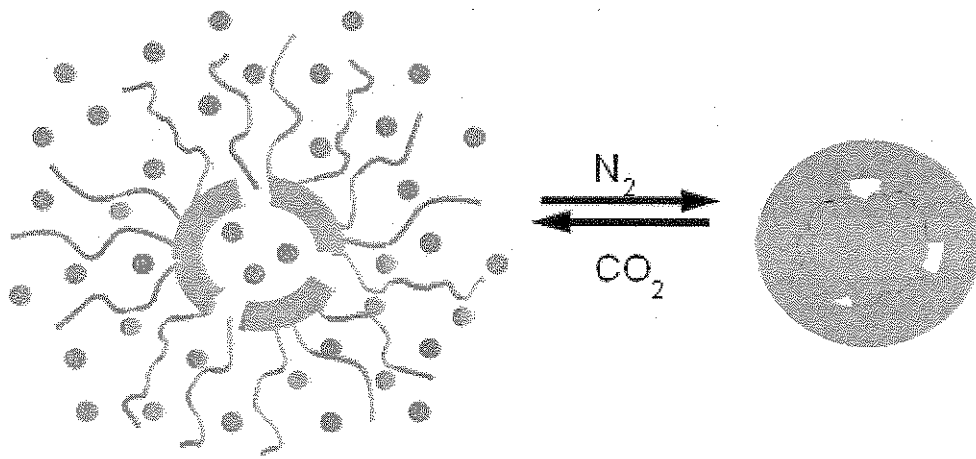
Abstract

Hollow Core-Shell Particles

The invention relates to hollow nanoparticles that can be used for gas delivery. The invention specifically relates to hollow core-shell particle having pores extending through its shell and a plurality of polymer chains that are grafted to the outer surface of the shell, wherein the polymer backbone of said polymer chains comprises monomer units that are functionalized with a group selected from amidines, guanidines, amino acid derivatives and heteroaromatic or non heteroaromatic heterocyclic groups, wherein the heterocycles comprise 1 to 3 nitrogen atoms. These particles can be filled with gas molecules that can be released by external triggers, such as for instance the presence of increased carbon dioxide concentrations. The invention further related to processes to make the particles and to fill them with gas molecules as well as their uses in applications which require oxygen release in otherwise difficult to address environment, such as skin care products or tissue or cell regeneration.

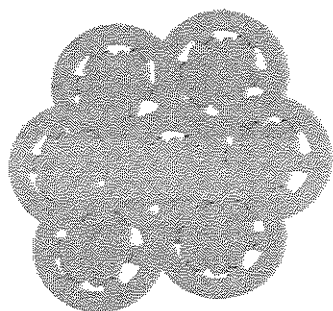
[Fig. 3]

[Fig. 1]

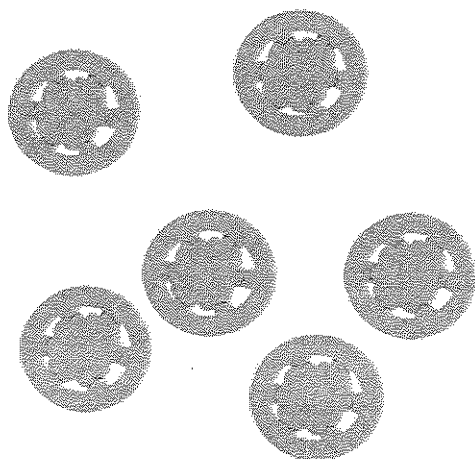


[Fig. 2]

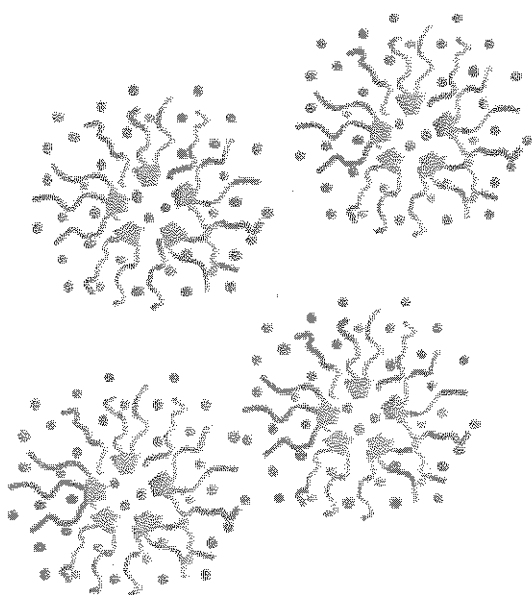
[Fig. 2a]



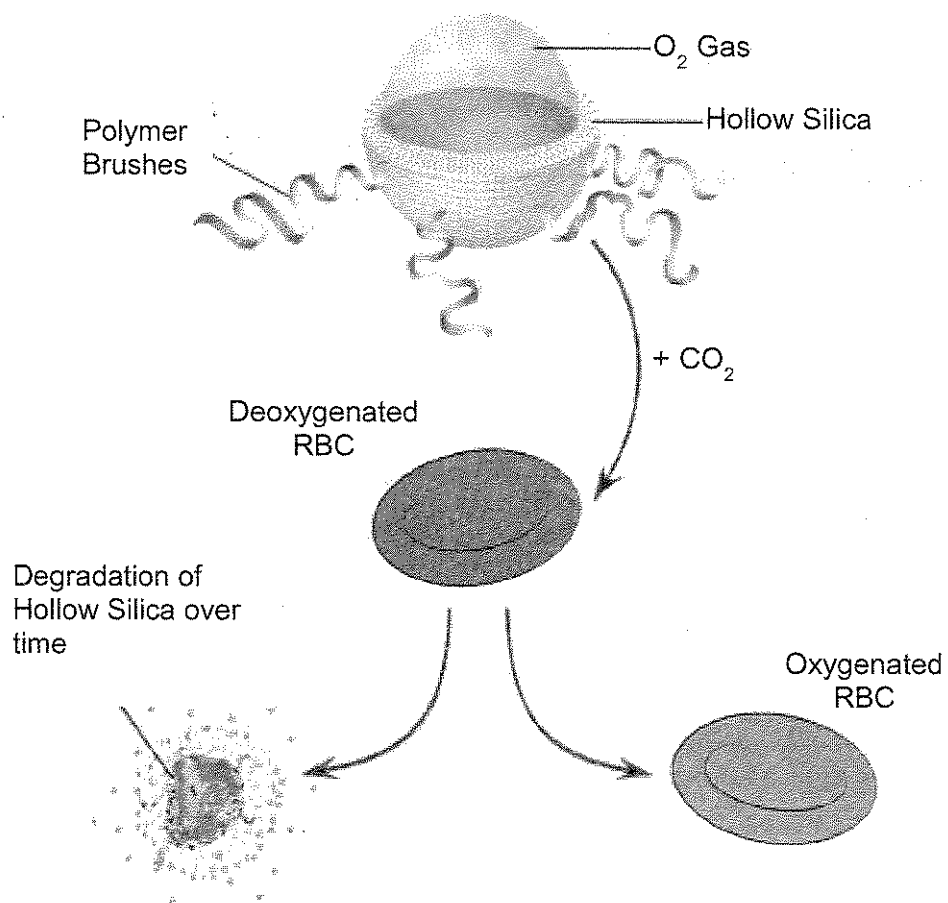
[Fig. 2b]



[Fig. 2c]

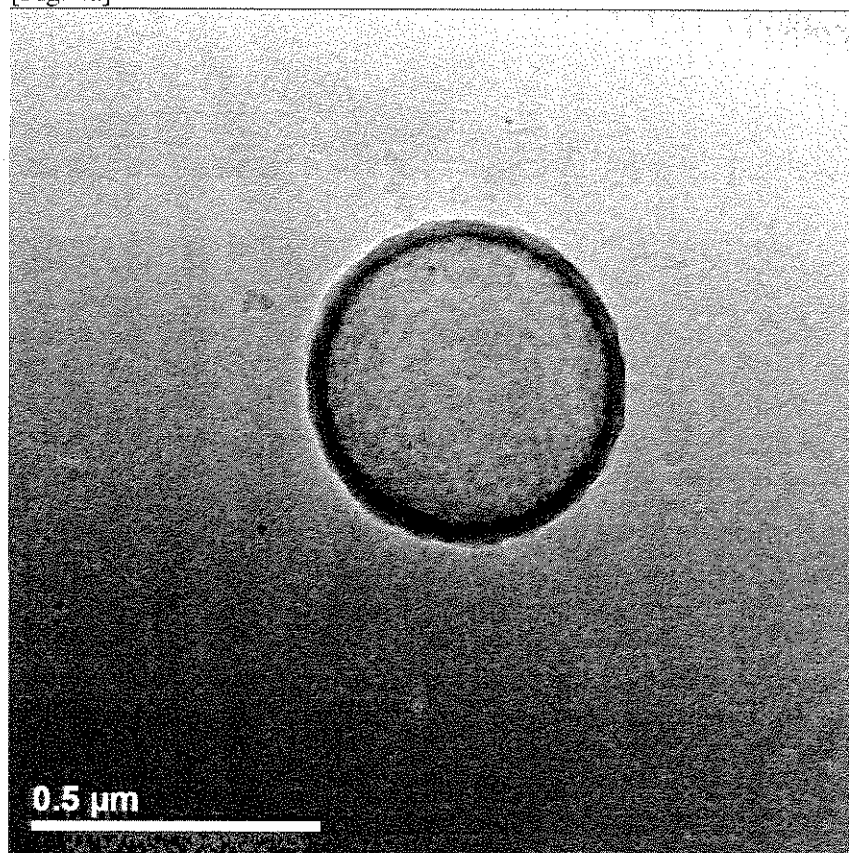


[Fig. 3]

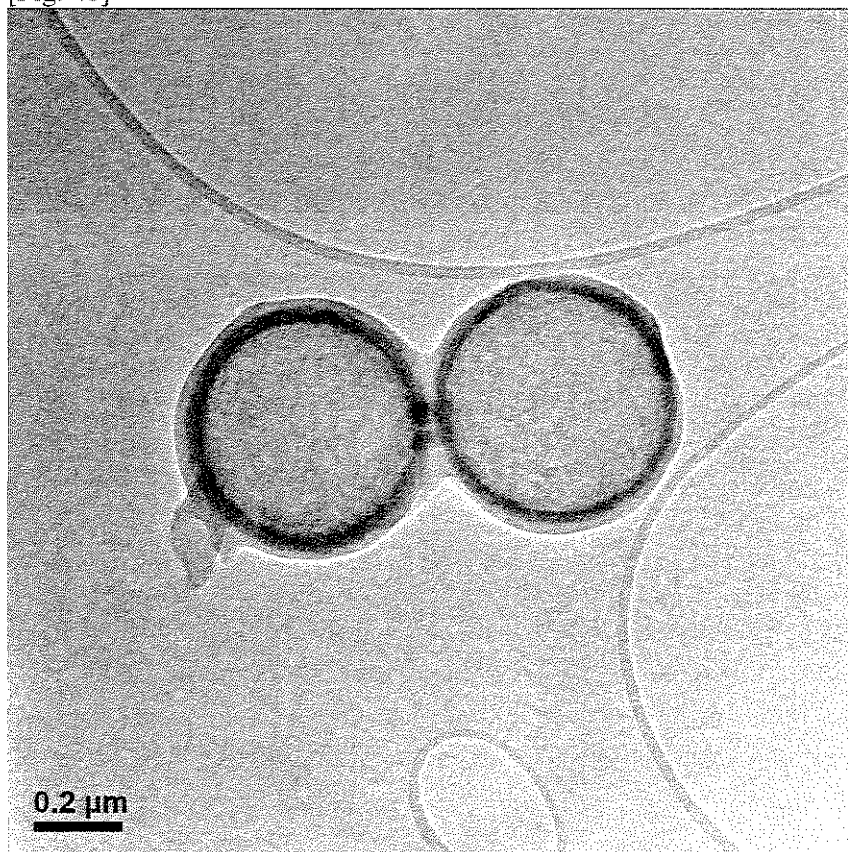


[Fig. 4]

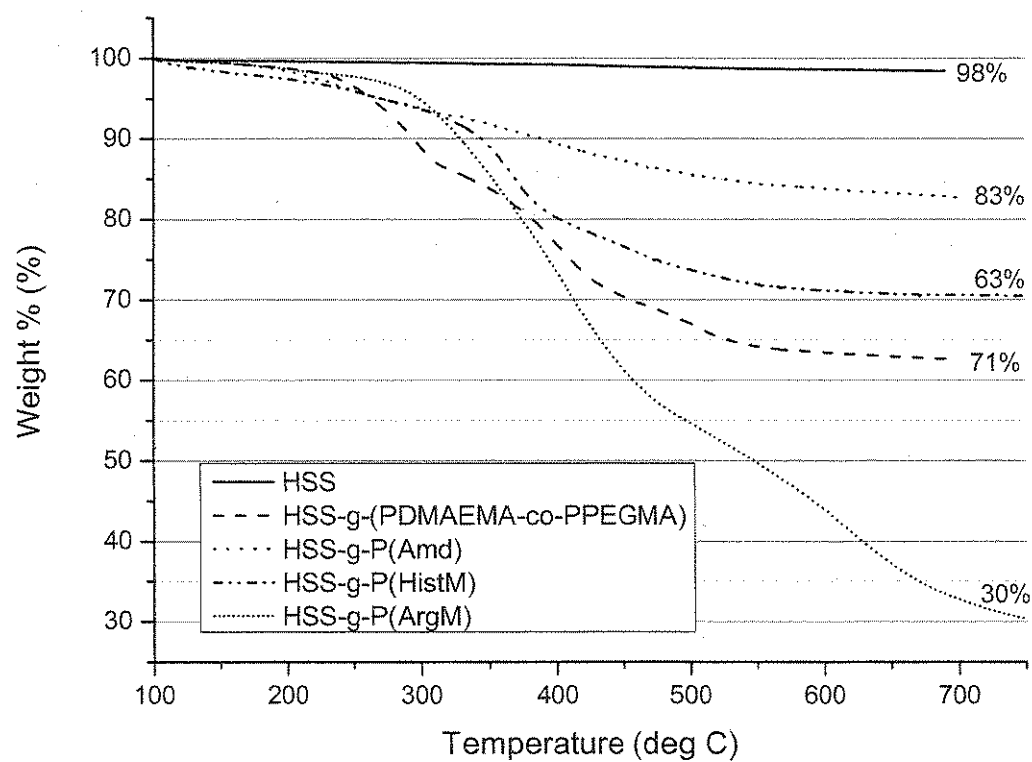
[Fig. 4a]



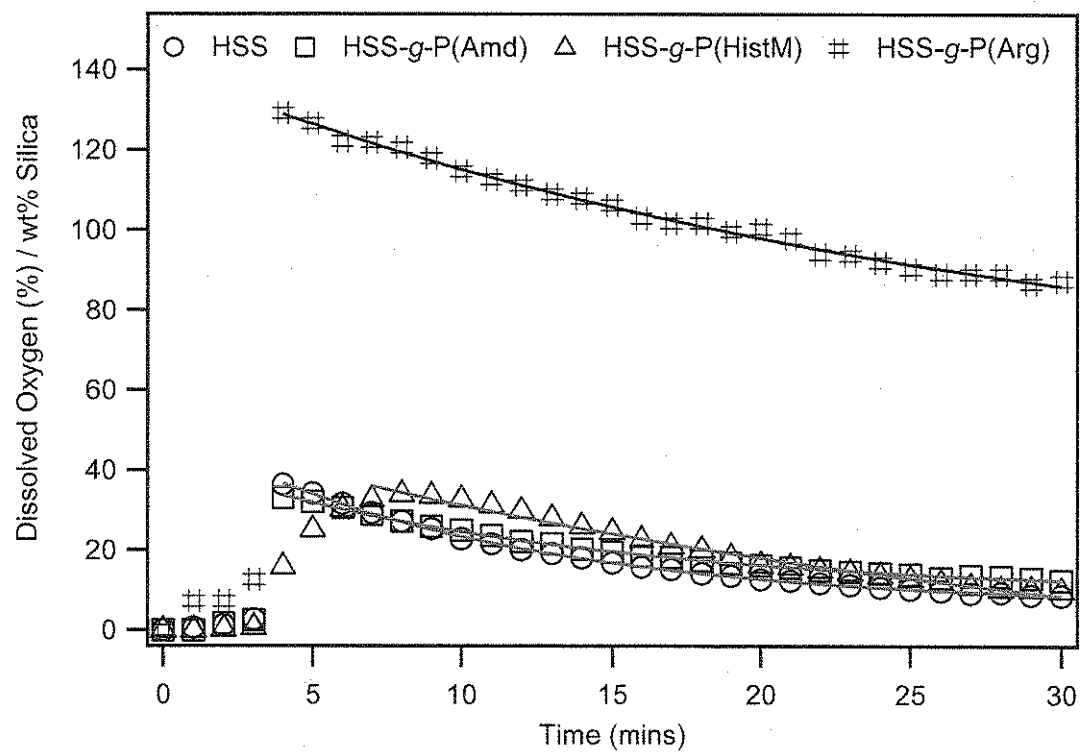
[Fig. 4b]



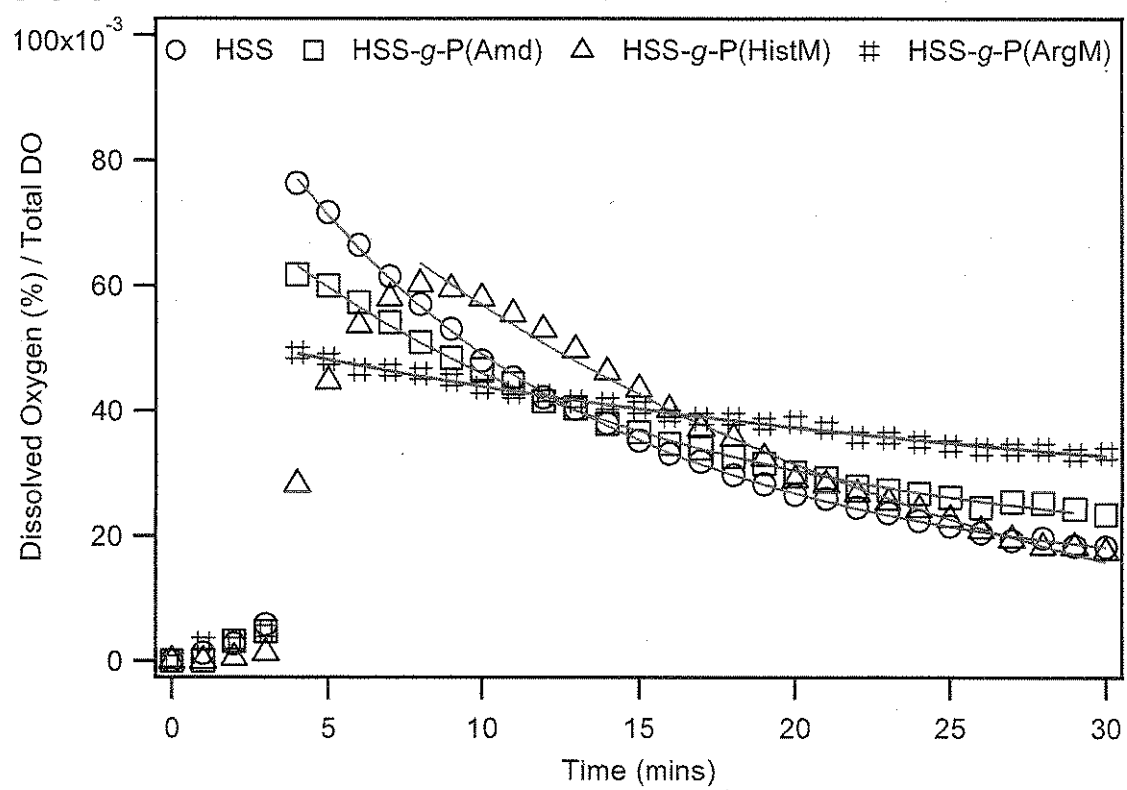
[Fig. 5]



[Fig. 6]



[Fig. 7]



[Fig. 8]

