(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 June 2009 (25.06.2009)

PCT

(10) International Publication Number
WO 2009/077908 A1

(51) International Patent Classification:
A61K 41/00 (2006.01) *A61K 47/48* (2006.01)
A61K 49/00 (2006.01) *A61K 9/51* (2006.01)
A61P 35/00 (2006.01) *A61K 47/36* (2006.01)
A61P 43/00 (2006.01)

(21) International Application Number: PCT/IB2008/055094

(22) International Filing Date: 4 December 2008 (04.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/007,690 14 December 2007 (14.12.2007) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2009/077908 A1

(54) Title: COLLOIDAL PARTICLE COMPRISING MULTIVALENT CYCLIC ANIONS

(57) Abstract: The present invention relates to colloidal particles comprising a polymeric polycation and cyclic system comprising at least one negative charge. The cyclic system, is preferably a photsensitizer. Surprisingly, colloidal particles successfully delivering photosensitizers to cells are provided, rendering the particles of the invention suitable for photodynamic therapy. Preferably, the particles of the present invention have a negative zeta potential, which is provided by a polyanion.

Colloidal Particle Comprising Multivalent Cyclic Anions

Technical Field

The present invention relates to the fields of polymer science, colloid science, polyelectrolyte chemistry, biomedical engineering and pharmaceutical sciences. More particularly, the present invention relates to a colloidal particle, to the use of the particle as a medicament, in particular in photodynamic therapy of diseases in humans and animals, in particular to a process of preparing the particle. The present invention also relates to cosmetic methods and products comprising colloidal particles.

Prior Art and the Problem Underlying the Invention

In US 2001/0051189 nanoparticles based on hydrophilic polymers as pharmaceutical form for the administration of macromolecules are disclosed. As is described in this reference, capsules form spontaneously in aqueous solutions comprising chitosan, the bioactive macromolecule and a the anion partner, typically tripolyphosphate (TPP). The incorporation of the bioactive macromolecule is thus achieved by a very simple and mild procedure which is effective for preserving the stability of the bioactive macromolecules.

Borges et al. International Journal of Pharmaceutics 299 (2005) 155-166 have further developed the system for incorporating the macromolecule ovalbumin, a protein. In this reference, a particle modification with sodium alginate is disclosed, resulting on an inversion of the particles zeta potential. Borges et al. suggest the use of their system for a mucosal vaccine delivery system limited to oral application.

WO 2007/042572 disclose the use of a colloidal system based on chitosan and heparin for the administration of the macromolecule heparin, so as to improve absorption of heparin through mucosal barriers and thus enable its administration by the oral route.

WO 2007/031812 disclose hydrophilic particles consisting of chitosan and an anionic polysaccharide, such as hyaluronic acid. Accordingly, in this reference it was shown that polysaccharides can successfully be used in the preparation of colloidal particles, and that a cross-linking counter anion, such as a tripolyphosphate, was surprisingly not required for producing stable nanoparticles.

In view of the prior art the problem underlying the present invention is to provide new applications of colloidal particles.

It is another objective of the present invention to provide colloidal particles made of components that differ from those described in the prior art, thus providing new, unexpected properties to the colloidal particles.

It is a particular objective of the present invention to provide colloidal particles suitable to encapsulate active principles other than macromolecules. In this context it needs to be understood that particles obtained by polyelectrolyte complexation, as described above, usually comprise a three dimensional network of the associated electrolytes and polyelectrolytes, distributed in the entire volume of the capsules. These capsules do not constitute a major barrier to the diffusion of water and other small molecules. Accordingly, one can expect that, in an aqueous solution, hydrophilic molecules present inside the capsules will diffuse out of the capsules without significant retention. Accordingly, because release cannot be controlled, the colloidal capsules reported in the prior art above are not expected to be useful for the encapsulation of bioactive molecules other than macromolecules.

It is also an objective of the present invention to provide a new form of administration for specific bioactive molecules which is based on naturally occurring components and/or which can be produced under mild conditions. Preferably, such a new form of administration preferably does not pose any risk to health of individuals to which the form of administration is administered.

It is a further objective of the present invention to provide a new delivery system for therapeutic agents which can be taken up by cells so as to deliver the therapeutic agent to the interior of the cells and preferably release it within the cells.

Furthermore, there are specific problems related to photodynamic therapy which are addressed by the present invention. In photodynamic therapy, a photosensitizer is typically administered systemically, so that overall sensitisation is observed in patients.

Further objectives and problems that are solved will become apparent from the description of the present invention herein below.

Summary of Invention

The present invention relates to colloidal particles comprising a polycation and a negatively charged, cyclic molecule. The present inventors successfully prepared colloidal particles in aqueous solutions; the particles stably and efficiently entrapping the cyclic molecules. A variety of such cyclic molecules can be entrapped, as mentioned further below. Examples are anionic derivatives of porphyrin and chlorin that have photophysical properties which can be exploited in medical and/or diagnostic applications, for example as detection agents (often called markers) or as photosensitizers. Interestingly, the anionic derivatives of porphyrin and chlorin that have photophysical properties, which are useful in photodynamic therapy, are successfully retained within the particles and taken up by cells exposed to the particles.

Without wishing to be bound by theory it is hypothesized that the presence of polar moieties, in particular multiple negative charges, account to some extent for the cyclic molecules' successful retention in and the controlled release from the colloidal particles according to the present invention. This finding allows for selecting specific molecules, such as bioactive principles, to be incorporated in the capsules of the invention.

The unexpected finding provides new applications for chitosan-based colloidal particles. It is now possible to immobilize molecules other than macromolecules inside the colloidal particles.

Accordingly, the present invention provides, in a first aspect a colloidal particle comprising at least one cationic polymer, and at least one negatively charged cyclic molecule. preferably, the colloidal particle has a negative zeta potential. Preferably, the cyclic molecule is multivalent negatively charged. The cyclic molecule is preferably selected from photosensitizer, including photosensitizer precursors, fluorescent compounds, phosphorescent compounds.

In a second aspect, the present invention provides a pharmaceutical composition, diagnostic tool and a cosmetic composition comprising the particles of the invention.

In a third aspect, the present invention provides the colloidal particle and/or the pharmaceutical composition of the invention for use as a medicament and/or in a diagnostic method.

In a fourth aspect, the present invention provides the particle and/or the pharmaceutical composition of the invention for use in the treatment and/or prevention of an inflammatory disease.

In a fifth aspect, the present invention provides the particle and/or the pharmaceutical composition of the invention for use in the treatment and/or prevention of an autoimmune disease.

In a sixth aspect, the present invention provides the particle and/or the pharmaceutical composition of the invention for use in the prevention and/or treatment of cancer.

In a seventh aspect, the present invention provides the particles and the pharmaceutical composition of the invention for use in photodynamic therapy.

In a eighth aspect, the present invention provides a method of treatment by photodynamic therapy the method comprising the steps of administrating to an individual in need thereof an effective amount of the colloidal particle or of the pharmaceutical composition of the invention, and exposing cells and/or a tissue of the individual to light, in particular a photo effective light.

Further aspects and preferred embodiments of the present invention are provided in the appended claims.

Brief Description of the Drawings

In the drawings,

Figure 1 shows the chemical structures of photosensitizers loaded into the colloidal particles of the present invention. Tetraphenylporphyrintetrasulfonate ($TPPS_4$) (**A**); Tetraphenylchlorintetracarboxylate ($TPCC_4$) (**B**); Chlorin e6 (Ce6) (**C**).

Figure 2 shows an electron microscopic picture of colloidal particles of a multitude of sizes of the present invention. The particles in the picture have a size ranging from approximately 50 to 300 nm.

Figure 3 A and B show the fluorescence of RAW mouse macrophages incubated without (A) and with (B) colloidal particles of the present invention. Figure 3 B shows that the particles were taken up by the cells from the fluorescence emanating from the cytoplasma around the nucleus.

Figures 4 A, B and C show the fluorescence of RAW mouse macrophages incubated with the colloidal particles of the present invention. In A, fluorescence stems from rhodamine isothiocyanate-labelled alginate (alginate-rhodamine) of the particles, in B fluorescence stems from carboxyfluorescin-labeled chitosan (chitosan-fluorescein) of the particles. Figure 4 C is an overlay of A and B.

Figure 5 shows cell survival of RAW mouse macrophages incubated with particles of the invention loaded with different photosensitizers shown in Figure 1 following exposure to irradiation. Blue squares indicate $TPPS_4$, Red circles: $TPCC_4$ and green triangles: Ce6. Black diamonds are control cells not exposed to photosensitizer-colloidal particles. Cells were exposed for 24h to particles (1/5 vol.) then irradiated with increasing doses of light.

Figure 6 shows the effect of incubation time with the particles and of particle concentration during incubation on cell survival following irradiation. The particles of the invention were loaded with Ce6 (C) (Figure 1) and applied to the culture medium at 1/5 vol (red squares) and 1/10 volume (green triangles). Black diamonds are control cells not exposed to the cells of the present invention.

Detailed Description of the Preferred Embodiments

The present invention provides colloidal particles based on a polycation, such as a chitosan, and a compound comprising a negatively charged, cyclic molecule. Preferably, the cyclic molecule is multivalent negatively charged. The term multivalent negatively charged refers to a molecule carrying two or more negative charges. The multivalent negatively charged, cyclic molecule is preferably selected from molecules having photophysical properties, in particular optically active compounds, such properties including phototoxicity (photosensitizers), fluorescence, and/or phosphorescence. Further preferred multivalent negatively charged cyclic molecueles are selected from photosensitizer precursors, and a combination including two or more of aforementioned. The colloidal particles preferably comprise one or more further negatively charged components. The particles of the present invention represent new vehicles for effectively delivering optically active compounds, such as photosensitizers, including photosensitizer precursors, fluorescent and/or phosphorescent compounds, for example markers.

The process of colloidal particle formation in solution is referred to in the prior art by different terms. For example, in US 2001/0051189 the expressions "ionic cross-linking" and "ionic gelation" are used. Borges et al. (2005), cited above, use terms as "precipitation" and "coacervation". Some of the above terms may suggest that covalent bonds are created during particle formation, which is not the case. Therefore, the present inventors prefer the term "polyelectrolyte complexation", which expresses accurately the fact that particles are formed through electrostatic forces.

Colloidal particles comprise at least one polyelectrolyte, which forms a three-dimensional network (matrix) occupying the volume of the particle, and, an oppositely charged electrolyte, which does not need to be polymeric, but which should carry sufficiently opposite charges so as to allow for the particle formation by electrostatic forces in aqueous solutions. As a polyelectrolyte, a polycation is typically used, such as a chitosan. In principle, however, these roles may be inversed, and an anionic polyelectrolyte could be used in combination with a multivalent cation.

A polymer, for the purpose of the present specification is a compound comprising 10 or more repeated moieties, such as more than 10 repeated saccharides, for example. An oligomer, for the purpose of the present invention, comprises 4 or more repeated moieties, the terms mono-, di and trimer thus being defined, for the purpose of the present invention, as is conventional.

Accordingly, in principle, the colloidal particles do not exhibit a core-shell structure as do many other known encapsulation delivery systems, for example those obtained by formation of oil in water emulsions, or for example liposomes. In core-shell encapsulation systems, the particle comprises a core of the substance to be delivered, which is retained by a capsule wall, which surrounds the substance to be delivered thus forming a shell that is basically impervious to the encapsulated substance.

In contrast, in the colloidal particles of the present invention, a matrix comprising at least one colloid is provided, with the substance to be delivered being retained in the matrix.

The surface characteristics of the particles of the present invention may be adjusted as desired. Preferably, the particles have a negative zeta potential. Desired surface characteristics may be obtained by "polyelectrolyte complexation", that is a polymeric substance attaches itself through electrostatic forces onto and/or partially into the surface of the particle and thus modifies the zeta potential, for example. Given the polymeric nature of the polymeric surface modulating substance, the latter will generally not substantially diffuse inside the particle but is predominantly located at the surface. Surprisingly, despite being of the same multivalent negative ionic character as the entrapped multivalent negatively charged cyclic molecules, the surface modulating polyanions do not liberate the multivalent negatively charged cyclic molecules from the colloidal system.

From the above the following terms will become apparent, which will be used herein to define the present invention. Accordingly, the "matrix" refers to the bulk of the particle volume, which comprises at least one polyelectrolyte and at least one electrolyte of opposite charge. The expression "matrix-forming" is attributed to components that help building up the matrix of the particle during particle formation. The "matrix-forming" components thus take part in the "polyelectrolyte complexation" that results in the particles of the present invention. Matrix-forming components are substantially evenly distributed in the matrix of the particle.

A cyclic molecule to be delivered preferably comprises at least two negative charges, which improves retention of the cyclic molecule within the matrix. The cyclic compound may or may not be matrix-forming. The particles of the present invention preferably comprise at least one polyanion, rendering the particle’s surface charge negative.

Further below, preferred constituents of the particles are indicated and their preparation is explained.

The colloidal particle of the present invention comprises a polycation, carrying a net positive charge under the conditions of particle formation. Conditions of particle formation vary depending on the different preparation steps and are preferably aqueous solutions with a pH of ≤ 9. The first preparation step preferably consists of creating the colloidal particles in the presence of the polycation (a cationic polymer).

Different types of polycations can be used in a particle of the invention. The cation is preferably a polymer. According to an embodiment of the present invention, the colloidal particles of the invention comprise, as a cationic polymer, a chitosan or a derivative of chitosan. For example, the colloidal particle comprises a mixture of two or more polycations, at least one of them being a chitosan and/or a chitosan derivative. Accordingly, the particle may comprise two or more polycations. In this case preferably at least one polycation is chitosan and/or a derivative thereof. The particles may also comprise chitosan, one or more derivatives of chitosan and, optionally, a further polycation.

Conditions of particle formation are preferably aqueous solutions with a pH ≤ 6, if the polycation is chitosan. If mixtures comprising chitosan and other polycations are used, the pH during particle formation is preferably ≤ 7.

The chitosans may differ in average molar mass, distribution of molar mass, degree of deacetylation, acetylation pattern, type of anionic counterion and purity. Regarding molecular size, chitosans with molar mass from 1’000 to 1’000’000 g/mol can be used in the particles of the invention. The lower end of this range (below molar masses of approximately 10’000 g/mol) includes molecules that are trivially referred to as oligochitosans and are characterized, with respect to chitosan of higher molar mass, by improved solubility in aqueous solutions at pH values higher than 6. Preferred molar masses of the chitosans used in the particles of the invention are from 1’000 to 10’000 g/mol and from 10’000 to 100’000 g/mol Typically, chitosans will be present in amounts exceeding 10% of the weight of the particles. Chitosans are produced from crustacean shells or by biotechnological processes from funghi. Commercial sources of chitosans are, e.g., Primex Ltd. (Iceland), Marinard Ltd. (Canada) or FMC Biopolymers (U.S.) as producers of crustacean-based chitosans, and Kitozyme Ltd. (Belgium) as producer for biotechnologically derived chitosan. Chitosans used in the particles of the invention can also be chemically modified, for example on their hydroxyl or on their amino functionality. Such derivatized chitosans can be used instead or in combination with unmodified chitosans or other polycations. Examples of moieties linked to the chitosan molecule are fluorescence markers such as fluorescein, anionic groups such as carboxymethyl, neutral synthetic small molar mass chains such as polyethylene glycol (PEG) chains and saccharides such as mono- or oligo-saccharides such as mannose and galactose. Modifications on the chitosan's amino functions can be executed in order to obtain secondary, tertiary or quaternary amines. The latter of being of special interest as a pH independent positive charge can be integrated in the chitosan molecule. Prominent derivatives are the trialkyl chitosans, such as trimethyl chitosan.

Of course, the skilled person may choose other polycations, which can be used together with or instead of a chitosan. Examples are polyethylene imine, polyethylene imine derivatives, poly(methylene-co-guanidine) and poly-L-lysine.

The particles of the present invention are beneficial over other processes of encapsulation due to the mild conditions under which particles can be formed and the possibility of adjusting particle size. Accordingly, particle formation may be conducted in aqueous solutions at ambient temperatures, for example 4°C to 40°C, preferably 10°C to 35, 15°C to 30°C, preferably at room temperature (25°C).

Without wishing to be bound by theory it is believed that, in an aqueous solution, at least one polycation interacts with at least one anionic species carrying at least two, but preferably a plurality (two or more) anionic charges, so that a cluster or matrix of polycation and anionic species is finally obtained, the cluster forming the particle of the present invention. By adjusting conditions, such as concentration of polycationic and anionic species, level of agitation of the aqueous solution, particle size can be adjusted.

The colloidal particle of the present invention thus comprises at least one anionic species, which is necessary to have particle formation through "polyelectrolyte complexation" as described above.

Surprisingly, the present inventors found that certain compounds that are to be delivered by and/or entrapped in the particle of the present invention may at the same time perform the function of negatively charged species enabling particle formation. This is indeed surprising, given that chitosan-based particles as those of the present invention were previously described only for the purpose of entrapping and/or delivering macromolecules, such as protein, RNA, DNA, heparin, and so forth. The present invention provides colloidal particles comprising a cyclic molecule, such as a cyclic bioactive compound, carrying negative charges as defined below, wherein said cyclic molecule, at the same, be partially or totally be employed as anionic species useful for particle formation by "polyelectrolyte complexation".

According to the present invention, the particle comprises a multivalent negatively charged, cyclic molecule. Preferably, the negatively charged cyclic molecule comprises a sequence of a plurality of 5 and/or 6-membered rings, said sequence of rings forming a circle so as to form the cyclic molecule. For the purpose of the present specification, the terms "5 membered ring" and "6-membered ring" refers to a ring having 5 and 6 ring atoms, respectively. An example for a 6-membered ring is benzene. The rings may be heterorings. An example of a 5-membered heteroring is 1H-pyrrol. The rings may be directly bound to each other by a covalent carbon-carbon single and/or double bond. Preferably, there is a linker connecting the rings, such as a carbon atom (for example, methylene) not forming part of any ring but being bound to two neighbouring rings so as to connect two neighbouring rings. The rings may be bound to the connecting atom by single and/or double bond. The connecting atom may be a heteroatom, for example selected from nitrogen and oxygen. In tetrapyrrole-based cyclic molecules, four pyrroles are interconnected by carbon bridges (methine or methylene).

According to a preferred embodiment, the cyclic molecule entrapped in the particles of the invention comprises a cyclic structure selected from chlorin, bacteriochlorin, porphin, corrin, corphin, phtalocyanin, benzoporphyrin and pyropheophoride, for example.

Accordingly, the negatively charged cyclic molecule may be selected from anionic chlorin derivatives, such as tetra(4-carboxyphenyl) chlorin and chlorin e6, for example.

Alternatively, or in addition, negatively charged cyclic molecule may, as part of the structure of the compound, comprise porphyrin (also named porphin or porphine). Accordingly, the multivalent negatively charged cyclic molecule may be selected from anionic porphyrin derivatives, such as meso-tetra (4-sulfonatophenyl) porphyrin, tetra(4-carboxyphenyl) porphyrin, oxyphor R0 (Pd-meso-tetra-(4-carboxyphenyl) porphyrin), oxyphor R2 (Pd-meso-tetra-(4-carboxyphenyl) porphyrin dendrimer), and protoporphyrin IX.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise corrin. Accordingly, the cyclic molecule may be selected from anionic derivatives of corrin.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise corphin. Accordingly, the cyclic molecule may be selected from anionic derivatives of corphin.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise phtalocyanin. Accordingly, the cyclic molecule may be selected from anionic derivatives of phtalocyanin.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise benzoporphyrin. Accordingly, the cyclic molecule may be selected from anionic derivatives of benzoporphyrin.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise bacteriochlorin. Accordingly, the cyclic molecule may be selected from anionic derivatives of bacteriochlorin.

Alternatively or in addition, the negatively charged cyclic molecule may, as part of the structure of the molecule, comprise pyropheophorbide. Accordingly, the cyclic molecule may be selected from anionic derivatives of pyropheophorbide.

The colloidal particle may comprise one or more different multivalent negatively charged cyclic molecules, for example selected from those mentioned above.

The multivalent negatively charged compound comprising a cyclic system preferably comprises a functional group constituting or transformable into an anionic moiety. For example, carboxylic acid groups are negative under specific pH conditions. Another example of a functional group suitable to provide one or more negative charges is the sulfonate group. Accordingly, the compound comprising a cyclic molecule preferably comprises at least one functional group selected from a carboxylate, a sulfonate and a phosphate, or combinations of two or more different groups. Preferably, the cyclic molecule comprises at least two, more preferably at least three functional groups selected from a carboxylate, a sulfonate and a phosphate group, and combinations thereof. In general, the cyclic molecule preferably carries two or more, 3, 4, 5, 6, 7, 8, 9 or more negative charges.

To a cyclic (basic) structure (for example, the chlorin, bacteriochlorin, porphin, etc. mentioned above), the one, two or more functional groups may be connected by way of a linker. Such linkers may be selected, for example, from alkandiyls, alkendiyls, aromatic rings such as benzenediyl, or other suitable linkers preferably comprising from 0 to 20, more preferably 1-10 carbon atoms. The linker may also be constituted of or comprise heteroatoms.

The cyclic structure may, besides the presence or absence of a linker, be further substituted. For example, it may be substituted with one or more C1-C10 alkyls, C2-C10 alkenyls and C4-C24 aryls, wherein said alkyl, alkenyl and aryl may comprise one or more heteroatoms and with the proviso that if the aryl is a C4 or a C5 aryl (for example, thiophene, purine, pyridine, pyrimidine), it comprises at least one heteroatom, independent from other possible heteroatoms, selected from O, S and N, in order to provide a substituent with aromatic properties. If the alkyl or alkenyl comprises three or more carbons, it may be cyclic and/or branched.

According to an embodiment, the cyclic structure, the at least one functional groups, the 0, one or more linkers, connecting the functional group to the cyclic structure, and the 0, one or more further substituents on the cyclic structure form the cyclic molecule of the invention.

According to a preferred embodiment, the cyclic molecule of the present invention is a C16-C200 hydrocarbon comprising one or more heteroatoms, preferably a C20-C100 hydrocarbon with 2-50 heteroatoms, and most preferably a C30-C50 hydrocarbon comprising 8-30 heteroatoms. Preferred heteroatoms are O, N, S, P, and halogen (F, Cl, Br, I) and may be the same or different. Heteroatoms may be part of the cyclic structures mentioned above, and are preferably provided in the functional groups as mentioned above, conferring a desired negative charge to the overall molecule under selected conditions and/or rendering the molecule soluble in water.

Preferably, the multivalent negatively charged, cyclic compound is a medicament, such as a drug, for example.

According to a preferred embodiment of the invention, the negatively charged, cyclic system is selected from molecules having photophysical properties, also referred to herein as photoactive or optically compounds, in particular photosensitizers, fluorescent compounds and/or phosphorescent compounds. While many photosensitizers may exhibit fluorescence and/or phosphorescence, not all fluorescent and/or phosphorescent compounds are useful as photosensitizers.

For the purpose of the present specification, the term "photosensitizer" refers to a compound, which increases photosensitivity of a tissue in which it accumulates. A "photosensitizer precursor", also referred to as "precursor" herein, is a compound that is converted to a photosensitizer when administered to a tissue. For the purpose of the specification, the terms "photosensitizer" and "precursor" may be used interchangeably. A photosensitizer, for the purpose of the present invention, includes photoxic compounds.

Specific examples of photosensitizers are those shown in Figure 1. Surprisingly, the exposure of cells to colloidal particles of the present invention comprising a photosensitizer increases the photosensitivity of these cells. This makes the particles of the present invention useful for all applications of photodynamic therapy (PDT). For example, the particles may be used in the treatment of human diseases such as rheumatoid arthritis, intraocular inflammation or cancer, where specific irradiation is applied to tissue containing the photosensitizer.

Fluorescent and/or phosphorescent compounds are useful in diagnostics and/or imaging. For example, due to their fluorescent and/or phosphorescent properties, their accumulation in a tissue of interested, for example a target tissue, may be monitored.

For the formation of the colloidal particle of the present invention additional, negatively charged species may be used, in particular one or more anions. For the purpose of the present specification, an anion comprises at least one, but preferably at least two, more preferably at least 3, 4, 5, 6, 7, 8, 9 or at least 10 negative charges in the conditions of particle formation.

For example, if the cyclic molecule only carries a single negative charge it may not allow particle formation by "polyelectrolyte complexation". Even if the cyclic system comprises two or more negative charges, a further anionic species may be useful and/or required for providing colloidal particles of desired properties. As it will be detailed further below, a polymeric anion is also useful for modulating the surface properties of the particle, in particular for providing particles having a negative zeta potential.

Accordingly, the particle of the present invention comprises a matrix-forming anion, which contributes to complexing during particle matrix formation. This anion may thus be referred to herein as "matrix-forming anion", as it will distribute across the three-dimensional network of the entire particle volume together with the polycation. Preferably, the matrix-forming anion comprises at least two negative charges. Preferably, the matrix-forming anion is a multivalent anion or a polyanion. The complexing anion may be provided, for example, by tripolyphosphate (TPP) as disclosed in US 2001/0051189.

The matrix-forming anion may also be selected from ATP (adenosine triphosphate), ADP (adenosine diphosphate), or derivatives thereof, and sulfate.

Matrix-forming polyanions may also be selected from polymeric substances, such as polyanions, for example alginate, hyaluronate, chondroitin sulfate, heparin, dextran sulfate, dermatan sulfate, carboxymethyl cellulose, carboxymethyl amylose, carboxymethyl dextran and derivatives of the before-mentioned molecules. Heparin and derivatives are disclosed in WO 2007/042572, which is expressly incorporated herein by reference. In particular, the section discussing heparin, starting from page 7, line 15 to page 8, line 17 is incorporated herein.

In WO 2007/031812, anionic polysaccharides are disclosed as matrix-forming polyanions, such as polysaccharides comprising carboxymethyl moieties, carboxy moieties and/or sulfate moieties. The entire reference of WO 2007/031812, and in particular the anionic complexing partners therein, are incorporated herein by reference.

The matrix-forming anion, can be any anion containing a plurality of negative charges at the pH value at which particle formation occurs. Specific examples of useful anions include the sulfate anion, oligophosphates such as tripolyphosphate (TPP), nucleoside triphosphate including adenosine triphosphate (ATP), nucleoside diphosphates including adenosine diphosphate (ADP), poly-acrylic acid, poly-methacrylic acid, chondroitin sulfate, alginate, hyaluronate, dextran sulfate, heparin, heparan sulfate, gellan gum, pectin, kappa, lambda and iota carrageenan, xanthan, exudate gums, carboxymethyl cellulose, carboxymethyl amylose, carboxymethyl dextran and derivatives thereof; sulfated, carboxymethylated, carboxyethylated or sulfoethylated varieties of glucans or xylans, glucan or xylan derivatives, glucosaminoglucans or glucosaminoglucan derivatives; proteins like collagen and keratose. All of these example anions are available from various commercial suppliers or can be synthesized by those skilled in the art using known methodology. Preferred anions are adenosine triphosphate, tripolyphosphate, alginate, hyaluronate, chondroitin sulfate, carboxymethyl cellulose and dextran sulfate. Most preferred are tripolyphosphate, chondroitin sulfate, adenosine triphosphate and alginate.

As is indicated above, the negatively charged cyclic molecule, for example a photosensitizer, preferably is a matrix-forming anion. The particle may comprise one or more different, further matrix-forming anions, such as those mentioned above.

The present invention may further comprise a surface-modulating polyanion. The surface-modulating polyanion may be used for modifying the surface properties of the colloidal particle of the present invention. Polyanions useful for modifying the surface properties have, in general, the purpose of providing a negative zeta potential to the particles. Particles with negative zeta potential tend less to aggregate in extracellular fluids. Furthermore, they do not exhibit the generally biologically incompatible properties of particles carrying many positive charges at their surface.

The polyanions for modifying surface properties of the particle are preferably located close to the surface of the particle, in proximity to the positive charges of the polycation of the particle so as to render the overall charges on the surface more negative than positive. As will be detailed further below, the surface modulating polyanion is preferably applied in a separate step, by adding particles having positive surface charges (a positive zeta potential) to a solution of the polyanion so as to create a negatively charged surface.

Interestingly, despite being of identical anionic character, the application of surface modulating polyanions to create a negative zeta potential does not liberate the multivalent negatively charged cyclic molecules.

Polyanions for modifying surface properties may be selected from basically the same polyanions mentioned above. For example, it may be selected from alginate, hyaluronate, chondroitin sulfate, heparin, dextran sulfate, dermatan sulfate, carboxymethyl cellulose, carboxymethyl amylose, carboxymethyl dextran, and derivatives of these polyanions. The particle may comprise one or more different, further surface modulating polyanions, such as those mentioned above.

Both, matrix forming anions and surface modulating polyanions used in particles of this invention can also be modified to carry ligands such as targeting ligands or non-interacting ligands or active principle or active principle precursor ligands. A targeting ligand is a moiety that binds to specific surface features of cells. Targeting ligands may address specific cell types and/or specific organelles within cells. Examples of targeting ligands are saccharides, liposaccharides, antibodies, cell adhesion molecules, signal peptides, cell uptake peptides, hormones and neurotransmitters. Furthermore, anions can be modified by moieties that do not specifically interact with cells. Such non-interacting moieties can be polyethylene glycol units of different molar mass with different termini. Examples of such termini are hydroxy and methoxy groups. Active principles are compounds that are supposed to provide a specific desired functionality in specific tissue or cells of an individual. For example, active principles include drugs, flavours, perfumes, neutraceuticals, more generally compounds and/or extracts providing a health benefit, nutrients, agrochemicals, such as pesticides, insecticides, and the like. Moreover, anions of this invention can be modified to carry targeting ligands linked to the anion via a spacer (linker) such as polyethylene glycol. Such modifications may be made using the carbodiimde reaction for linking carboxyl and amine functionalities to form amide bonds. For example, a carboxyl group of the polyanion can be reacted with a terminal amine of a polyethylene glycol molecule; a bifunctional polyethylene glycol molecule can be reacted with both a targeting ligand and an anion. These reaction pathways are known under the term PEGylation.

Preferably, targeting ligands and non-interacting ligands are attached to polyanions. More preferably, such targeting ligands and non-interacting ligands are attached to the surface modulating polyanion. Active principle or active principle precursor ligands are attached to matrix-forming anions or to surface modulating anions. Anions might carry a plurality of different ligands.

Depending on conditions under which the particles of the invention are produced (concentration, agitation, pH, temperature), colloidal particles in the nanometer to micrometer ranges can be produced. Preferred colloidal particles of the invention are nanoparticles having an average diameter of between about 10 and 1000 nm, preferably 20 to 500 nm.

The size and size-distribution of the particles of the present invention may most accurately be determined by taking representative electron microscopic photographs and by measuring the particle diameters from the photographs.

Specific sizes of particles may be produced, as indicated above, by adjusting the conditions and parameters of the particle formation. Particles may also be subjected to fractionation, for example filtration, so as to obtain specific particle size fractions of specific distributions adapted for a particular use or application of the particles. For example, the particles may have an average diameter that is smaller than 100 nm, in the range of 100-300nm, 200-500nm or 300-600nm, as preferred for a specific application.

When in an aqueous solution, the colloidal particle's matrix does not, or substantially does not constitute a major barrier to the diffusion of water or other small compounds. The surface properties of the particles may be adjusted by the use of specific polyanions and/or polycations and by adjusting their concentration during preparation, for example. The particle can also be regarded as a gel, which, in aqueous solution binds and incorporates water.

The particles may be dried, for example by freeze-drying or by other drying techniques such as spray drying, for example.

With respect to dry weight, the particle may comprise:

10-60 wt.%, preferably 10-40 wt.%, more preferably 20-30 wt.% of polycation, such as chitosan; 5 to 40wt.%, preferably 5-30 wt.%, more preferably 10-20 wt.% of the multivalent negatively charged cyclic compound (for example a photosensitizer); 5 to 90wt%, preferably 20-80 wt.%, more preferably 40-70 wt.% of other anionic species (polyanions), including surface modulating polyanions and matrix-forming anions different from the negatively charged compound comprising the multivalent negatively charged cyclic system.

Preferably, the particles of the present invention have a negative zeta potential. A negative zeta potential is determined by electrophoretic mobility measurements and represents a net negative surface charge of the particle.

The present invention provides a method for producing colloidal particles of the invention. Colloidal particles of the invention can be obtained readily by drop-wise addition of an aqueous solution comprising one component of the particles to an aqueous solution containing another component of opposite charge and agitation. No particular attention needs to be paid to the size of the droplets or the flow rate of addition of the first solution to the second solution. Formation of the particles of the present invention occurs spontaneously by polyelectrolyte complexation of the system's anionic components and polycation (e.g. chitosan). Particle formation is visualized in the so-called "Tyndall effect" that can be seen by the human eye. The solvent system for the component solutions can be water or aqueous salt solutions. Conditions of pH can be varied depending on the type of polycation (e.g. the type of chitosan) used and can cover physiological pH ranges. Chitosans of molar masses above approx. 10,000 g/mol require slightly acidic pH values, preferably between pH 4.0-6.6, whereas chitosan of molar mass below 10,000 g/mol have a wider pH range in complex formation, pH 4.0-7.5.

Within certain limits, water-miscible solvents can be present, e.g., alcohols such as methanol, ethanol, 2-propanol, or N-butanol, can be present at concentrations of up to about 20% (v/v).

Chitosan polyelectrolyte complex formation has been extensively described in Berger et al. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. J. Pharm. Biopharm. 57 (2004), 35-52 and Agnihotri et al. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. Journal of Controlled Release 100 (2004), 5-28.

According to an embodiment, the colloidal particle of the invention is obtained by polyelectrolyte complexation in aqueous solution conducted at a temperature in the range of

1°C to 100°C and at a pH in the range of 4 to 9. Preferably, the temperature is 5-50°C, most preferably 10 to 35°C.

The number and order of steps that are performed to produce particles of the invention can be varied. For example, a solution containing one or more anions and the multivalent negatively charged cyclic system may be combined as described above with a solution of a chitosan or of a mixture of different polycations comprising chitosan. Amounts of components combined are chosen such that particles with negative zeta potential result from polyelectrolyte complex formation. Another method is to combine a solution comprising the multivalent negatively charged cyclic molecule and, optionally, an anion with a solution comprising a chitosan, or a mixture of different polycations comprising chitosan, such that colloidal particles of positive zeta potential are obtained. If necessary, an excess of uncomplexed chitosan can be removed by processes such as dialysis, ultrafiltration and centrifugation. Thereafter, the dispersion of particles of positive zeta potential is combined with a solution comprising one or more surface modulating polyanions, forcing conversion of the particles with positive zeta potential to particles with negative zeta potential. It is noted that the two or more polyanions that are incorporated in the final particles may be the same or may be different. A variation of the previous method is to produce a first dispersion of colloidal particles with positive zeta potential by combining a solution of chitosan and a solution of one or more polyanions. After removal of excess chitosan, should there be an excess, the first dispersion is combined with a solution comprising a multivalent negatively charged, cyclic molecule as defined above, and, if desired, one or more anions to produce a second dispersion, still of positive zeta potential. This second dispersion is then added to a solution of one or more surface modulating polyanions to force conversion to particles with negative zeta potential.

Since particle formation occurs in aqueous solutions, including aqueous solutions comprising other solvents in addition to water as indicated above, the polycations and anions described herein are preferably soluble in such aqueous solutions. The requirement of solubility is met, if at the temperature of particle formation sufficient amounts of polycation or anion, for example a polysaccharide, are dissolved in a specific solution so that particle formation through electrostatic forces can occur.

It is noted that additional components can be added during or after particle formation. Examples of such additional components are multivalent cations such as calcium, uncharged polymers such as polyethylene glycol, or uncharged saccharides. Additional components may also include one or more biologically active substances, in particular bioactive principles as mentioned above. Such biologically active substances may be any biologically active substance, including small-molecule drugs or pro-drugs and therapeutic or otherwise biologically active peptides or proteins, provided that they are soluble in aqueous solutions at concentrations exceeding the concentrations at which they are therapeutically active or exert their other biological activity. Specific examples of such biologically active substances are NSAIDs, preferably NSAIDs belonging to the classes of salicylates, aryl alkanoic acids, 2-aryl propionic acids, *N*-aryl anthranilic acids, pyrazolidine derivatives, oxicams, coxibs and sulphonanilides.

The particles of the present invention are useful in photodynamic therapy in human or animals, in particular of mammals.

The particles are useful in the prevention and/or treatment of a human disease, for example in the prevention and/or treatment of cancer, and/or of a proliferative disease and/or an inflammatory disease. For example the particles are useful for the prevention and/or treatment of intraocular inflammation and/or inflammatory bowel disease.

The particles may also be used in the prevention and/or treatment of rheumathoid arthritis.

According to an embodiment, the particles of the present invention are used in the prevention and/or treatment of an autoimmune disease.

The particles of the invention may be used for the prevention and/or treatment of a disease selected from addison's disease, alopecia reata, ankylosing spondylitis, antiphospholipid antibody syndrome, autoimmune hemolytic anemia, autoimmune hepatitis, bullous pemphigoid, coeliac disease, Crohn’s disease, dermatomyositis, diabetes mellitus type 1, goodpasture's syndrome, graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, lupus erythematosus, multiple sclerosis, myasthenia gravis, pemphigus vulgaris, pernicious anaemia, polymyositis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, Sjögren's syndrome, temporal arteritis, ulcerative colitis, uveitis, vasculitis, Wegener's granulomatosis, and combinations of two or more of the aforementioned.

In photodynamic therapy, a tissue is irradiated with light of specific intensity and at a wavelength, which is absorbed by the photosensitizer. Without wishing to be bound by theory, the absorption of light by the photosensitizer results in cytotoxicity, probably associated with the occurrence of cytotoxic substances that induce cell death. The irradiation wavelength thus depends on the specific photosensitizer that is used. The skilled person will thus choose the wavelength according to the photophysical / optical properties of the specific photosensitizer employed. The term "photo effective light", for the purpose of the present specification, indicates that the light wavelength and intensity is adjusted so as to obtain a desired cytotoxic, fluorescent and/or phosphorescent effect. In general, the irradiation intensity is determined from the properties of the photosensitizer and the tissue to treat. The administered dose of photosensitizer and light is adjusted so as to obtain an optimal desired effect.

The particles of the present invention may be administered in any suitable way of administration, such as enterally and/or parenterally. For example, the particles may be locally injected into target tissue, e.g. by intra tumoral, intra vitriol, intra articular or by sub-cutaneous injection.

The particles may be provided in the form of a pharmaceutical composition, that is, a formulation suitable to be administered to an individual. Such formulations may comprise stabilisers, buffers, fillers, for example. The formulation may be liquid or solid, for example in the form of a dried powder or a tablet. The skilled person will select suitable formulations in dependence of the condition to be treated and the way of administration.

The present invention as described herein may be modified while still falling into the general scope of the inventive principle. For example, as already indicated above, charges of the constituents of the particles may be inversed without changing the principle of the present invention. For example, instead of a chitosan, a negatively charged polymer may be used and particle formation (“polyelectrolyte complexation") may be obtained by addition of a multivalent cationic molecule, which does not necessarily need to be polymeric (thus replacing tripolyphosphate, for example). In this case, also the entrapped cyclic system has to carry positive charges.

The invention is further elaborated by the following examples. The examples are provided for purposes of illustration to a person skilled in the art and are not intended to be limiting the scope of the invention as described in the claims. Thus, the invention should not be construed as being limited to the examples provided, but should be construed to encompass any and all variations that become evident as a result of the teaching provided herein.

Examples

Particle forming materials:

Chondroitin sulfate type A and TPP were purchased at Sigma-Aldrich (Sigma-Aldrich, Germany) and used without further purification. Hyaluronate of molecular weight of approx. 200 kg/mol was purchased at Lifecore (Lifecore, U.S.). Alginate of low and middle viscosity was obtained from Medipol (Medipol Ltd., Switzerland). Chitosan was purchased at Sigma-Aldrich (Sigma-Aldrich, Germany) and purified by Medipol (Medipol Ltd., Switzerland). Reduced molecular weight chitosan, i.e. molecular weight of approx. 5 kg/mol, was obtained from Medipol (Medipol Ltd., Switzerland). Chitosan-fluorescein and alginate-rhodamine were obtained from Medipol (Medipol Ltd., Switzerland).

Entrapped substances:

Pd-meso-tetra-(4-carboxyphenyl) porphyrin (commercial name Oxyphor R0) and Pd-meso-tetra(4-carboxyphenyl) porphyrin dendrimer (commercial name Oxyphor R2)were purchased at Oxygen Enterprises Ltd. (PA, USA). Meso-tetra (4-sulfonatophenyl) porphyrin tetra and sodium salt hydrate ($TPPS_4$) were purchased at Sigma-Aldrich (Sigma-Aldrich, Germany). Chlorin e6 (Ce6) and tetra(4-carboxyphenyl) chlorin ($TPCC_4$) were purchased from Frontier Scientific Inc. (UT, USA).

Example 1: Preparation of colloidal particles with negative zeta potential containing chitosan, Oxyphor R0 and chondroitin sulfate

12.2 mg Oxyphor R0 was dissolved in 57 mL water at pH 7. At room temperature, this solution was added drop-wise under agitation to a solution of 20.0 mg chitosan (subjected to purification prior to use) in 32 mL aqueous HCl at approx. pH 5. Opalescence appeared after the first added drops and became increasingly intense. After 1h of gentle agitation, the dispersion was filtered through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany) and dialyzed against water using a 0.05 µm hollow fiber module (KrosFlo module, polysulfone membrane, Spectrum Laboratories, U.S.). A milky, opalescent dispersion with visible Tyndall effect resulted, which remained unchanged after filtration through 1.2 µm and 0.8 µm filters (mixed cellulose ester membrane, Sartorius, Germany). Zeta potential was higher than +10mV. 20mL of the dispersion containing particles of positive zeta potential was added drop-wise to a solution of 20 mL of 0.05% chondroitin sulfate sodium salt in water at pH 7. A milky, opalescent dispersion with visible Tyndall resulted, which remained unchanged after filtration through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany). The zeta potential was measured at less than -10mV.

Example 2: Preparation of colloidal particles with negative zeta potential containing chitosan, $TPPS_4$ and tripolyphosphate and hyaluronic acid sodium salt

8.40 mg $TPPS_4$ and 2.23 mg tripolyphosphate were dissolved in 35 mL water at pH 7. At room temperature, this solution was added drop-wise under agitation to a solution of 20.0 mg chitosan (subjected to purification prior to use) in 32 mL aqueous HCl at approx. pH 5. Opalescence appeared after the first added drops and became increasingly intense. After 1h of gentle agitation, the dispersion was filtered through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany) and dialyzed against water using a 0.05 µm hollow fiber module (KrosFlo module, polysulfone membrane, Spectrum Laboratories, U.S.). A milky, opalescent dispersion with visible Tyndall effect resulted, which remained unchanged after filtration through 1.2 µm and 0.8 µm filters (mixed cellulose ester membrane, Sartorius, Germany). Zeta potential was higher than +10mV. 20mL of the dispersion containing particles of positive zeta potential was added drop-wise to a solution of 20 mL of 0.05% hyaluronic acid sodium salt in water at pH 7. A milky, opalescent dispersion with visible Tyndall resulted, which remained unchanged after filtration through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany). The zeta potential was measured at less than -10mV.

Example 3: Preparation of colloidal particles with negative zeta potential containing oligochitosan, Ce6 and chondroitin sulfate and sodium alginate

At room temperature, a solution of 45 mL of 0.1% chondroitin sulfate sodium salt in water at pH 7.0 was added drop-wise under mechanical agitation to a solution of 900mL of 0.025%

oligochitosan (M_n approx. 4500 g/mol, M_w approx. 6000 g/mol) in aqueous HCl at pH 5.5. Opalescence appeared after the first added drops and became increasingly more intense. After 1h of gentle agitation, the dispersion was filtered through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany), crossflow-dialyzed against water using a 0.05 µm hollow fiber module (KrosFlo module, polysulfone membrane, Spectrum Laboratories, U.S.) and concentrated to 300 mL. At room temperature, to 32 mL of this dispersion was slowly added under gentle stirring a solution of 4.9 mg of chlorin e6 (Ce6) in 35 mL of water at approx. pH 5, followed by 1h of agitation. The resulting milky, opalescent dispersion had visible Tyndall effect, which remained unchanged after filtration through 1.2 µm and 0.8 µm filters (mixed cellulose ester membrane, Sartorius, Germany). Zeta potential was found to be greater than +10mV. Subsequently, at room temperature, the dispersion was added to 70 mL of 0.05% sodium alginate (low viscosity type) in water at pH 7, followed by 1h of agitation. The resulting milky, opalescent dispersion had visible Tyndall effect that resisted filtration through 1.2 µm and 0.8 µm filters (mixed cellulose ester membrane, Sartorius, Germany). Zeta potential was less than -10mV.

Example 4: Preparation of colloidal particles with negative zeta potential containing chitosan-fluorescein, tripolyphosphate and sodium alginate-rhodamine

2.23 mg tripolyphosphate were dissolved in 35 mL water at pH 7. All following process steps were executed under protection from intense light. At room temperature, this solution was added drop-wise under agitation to a solution of 20.0 mg chitosan-fluorescein (subjected to purification prior to use) in 32 mL aqueous HCl at approx. pH 5. After 1h of gentle agitation, the dispersion was filtered through a 1.2 µm filter (mixed cellulose ester membrane, Sartorius, Germany) and dialyzed against water using a 0.05 µm hollow fiber module (KrosFlo module, polysulfone membrane, Spectrum Laboratories, U.S.). Zeta potential was higher than +10mV. 20mL of the dispersion containing particles of positive zeta potential were added drop-wise to a solution of 20 mL of 0.05% alginate-rhodamine sodium salt in water at pH 7. The zeta potential was measured at less than -10mV.

Example 5: Particles of the Invention in Photodynamic Therapy

In other to test biological effect and efficacy of the colloidal particles obtained in the preceding examples, cells were exposed to the particles and uptake of the particles was determined by fluorescence microscopy. Furthermore, it was checked if the uptake of the particles would render the cells photosensitive.

Material and Methods: Zeta potential determination

The nanoparticle dispersions were measured without further treatment of the sample by the means of Malvern Zetasizer ZS90 device (Malvern Ltd., Renens, Switzerland). From the same producer, a disposable electrophoretic measurement cell was employed (DTS1060C), The device settings for measurements in water at 25°C were applied.

Material and Methods: Fluorescence microscopy

RAW mouse macrophages cells were grown on glass slides until 25% confluent. The culture media were replaced with fresh medium containing particles diluted 1/5 in the medium and the cells were exposed to the particles for 24h. Thereafter, cells were washed with PBS. For DAPI staining, slides were incubated for 30 min in 3μM 4',6'-diamidino-2-phenylindolylhydrochloride (DAPI, Roche Diagnostics, Mannheim, Germany). Slides were mounted in PBS and analyzed under a fluorescence microscope (Axioplan2, Carl Zeiss, Feldbach, Switzerland) with filters set at 365 ± 5 nm excitation light (BP 365/12, FT 395, LP 397) for DAPI, 470 ± 20 nm excitation light (BP 450-490, FT 510, BP 515-565) for fluorescein and 535 ± 25 nm excitation light (BP 510-560, FT 580, LP 590) for photosensitizers and rhodamine.

Material and Methods: Phototoxicity experiments

Cells were grown in 96-well cell culture plates (Corning, NY) until 75% confluent. The culture media were replaced with fresh medium containing nanoparticles diluted 1/5 in the medium and the cells were exposed overnight to the nanoparticles. Then, the media were replaced with DMEM without phenol red containing 5% FCS and cells were irradiated at 652 nm using a diode laser (Applied Optronics, South Plainfield, NJ) coupled to a frontal diffuser (Medlight SA, Ecublens, Switzerland), at an irradiance of 20 mW/cm^2 and increasing light doses (up to 15 J/cm^2). Experiments were performed in triplicate wells. Cell viability was evaluated 24 h later using the MTT test. Briefly, 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT, Merck) was added at 250 μg/mL and incubation was continued for 2 h, then the cell culture supernatants were removed. The cell layers were dissolved in iPrOH/0.04N HCl and the absorbances were measured at 540 nm in a multiwell -

plate reader (iEMS Reader MF, Labsystems, Bioconcept, Switzerland) and compared to the values of control cells incubated with the nanoparticles but without laser irradiation.

For kinetic studies, cells were grown in 96-well cell culture plates until 75% confluent. The culture media were replaced with fresh medium containing particles diluted 1/5 or 1/10 in the culture medium and the cells were exposed to the nanoparticles for time-course ranging from 30 min to 24 h. Then, the media were replaced with DMEM without phenol red containing 5% FCS and cells were irradiated at 652 nm using a diode laser at an irradiance of 20 mW/cm^2 and light dose of 2 J/cm^2. Experiments were conducted in triplicate wells. Cell viability was evaluated 24 h later using the MTT test, as above.

Results:

Fluorescence microscopy studies of RAW mouse macrophages incubated 24h with Hynosphere NPs surface-modulated with hyaluronic acid and loaded with TPPS$_4$ (Example2) revealed that the porphyrin-associated fluorescence accumulated as red fluorescent spots in the cytoplasm of cells but not in the nucleus as can be seen in Figures 3 A and B. Figure 3 B. Figure 3 A shows the control, that is cells incubated without particles, while Figure 2 B shows cells incubated with particles. DAPI staining of nucleus revealed neither obvious cell toxicity nor nuclear fragmentation.

The cell uptake of the colloidal particles was studied using empty colloidal particles composed of material covalently modified with fluorescence reporters (chitosan-fluorescein and alginate-rhodamine) (Example4). Photoactive multivalent negatively charged cyclic molecules, as presented through the examples 1 to 3, would interfere with the fluorescence of the colloidal particle composing fluorescence-marked material and are therefore for this study not incorporated. Figures 4 A – C show fluorescence microscopy study of RAW mouse macrophages incubated 24h with the above-mentioned colloidal particles. Figure 4 A shows fluorescence of rhodamine (from alginate-rhodamine), Figure 4 B shows fluorescence of fluorescein (from chitosan-fluorescein) and Figure 4 C shows the merged image of Figures 4 A and B. The results indicate that the fluorescence of both reporter fluorophores can be found inside cells suggesting that intact nanoparticles can be taken up by cells.

Photosensitivity induced by photosensitizer-loaded colloidal particles was evaluated after 24 h of incubation in cells exposed to colloidal particles loaded with the same amount of the three different photosensitizers. The result is shown in Figure 5. All the nanoparticles induced phototosensitivity, but Ce6-loaded nanoparticles were the most active. For these nanoparticles, light dose inducing 50% of mortality (LD_{50}) was 0.5 J/cm^2, while the LD_{50} was 2 J/cm^2 for $TPCC_4$-loaded nanoparticles and 12 J/cm^{2}for $TPPS_4$-loaded nanoparticles. This hierarchy of photo-efficiency is comparable with the photo-efficiency of the free photosensitizers.

The kinetic of cell response to Ce6-loaded nanoparticles was also studied. Cells were exposed to Ce6-loaded nanoparticles for different periods of time and irradiated at 2 J/cm^2. Figure 6 indicates that 75% of effect is reached already after only 3 h of cell exposure.

Claims

1. Colloidal particle of negative zeta potential comprising at least one cationic polymer, and at least one negatively charged cyclic molecule.

2. The colloidal particle of any one of claims 1, wherein the cyclic molecule is selected from photosensitizers, photosensitizer precursors, fluorescent compounds, phosphorescent compounds and combinations of two or more of these.

3. The colloidal particle of claim 1 or 2, wherein the negatively charged cyclic molecule comprises a sequence of a plurality of 5 and/or 6-membered rings, said sequence of rings forming a circle so as to form the cyclic molecule.

4. The colloidal particle of any one of claims 1 - 3, wherein the negatively charged cyclic molecule comprises a structure selected from a porphyrine, chlorin, bacteriochlorin, corrin, phtalocyanin, benzoporphyrin, pyropheophrobide, and combinations of two or more of these.

5. The colloidal particle according to any of the preceding claims, wherein negative charges of the cyclic molecule stem from one or more of the groups selected from carboxylate, sulfonate and/or phosphate.

6. The colloidal particle of claim 1, comprising, as a cationic polymer, a chitosan or a derivative of chitosan.

7. The colloidal particle according to any one of the preceding claims, having an average diameter of about 10 to 600 nm.

8. The colloidal particle according to any one of the preceding claims, which is obtained by polyelectrolyte complexation in aqueous solution conducted at a temperature in the range of 1°C to 100°C and at a pH in the range of 4 to 9.

9. The colloidal particle of any one of the preceding claims, wherein the polycation forms a three-dimensional matrix across the volume of the particle with the cyclic system being dispersed within the matrix.

10. The colloidal particle of any of the preceding claims, comprising one, two or more different polyanions.

11. The colloidal particle according to any one of the preceding claims, comprising a surface-modulating polyanion.

12. The colloidal particle according to any one of the preceding claims, comprising a matrix-forming anion.

13. A pharmaceutical composition comprising the particle of any one of claims 1-12.

14. A diagnostic tool comprising the particle of any one of claims 1-12.

15. A cosmetic composition comprising the particle of any one of claims 1-12.

16. The colloidal particle according to any one of claims 1-12 and the pharmaceutical composition of claim 13 for use as a medicament.

17. The colloidal particle according to any one of claims 1-12 for use in a diagnostic method.

18. The colloidal particle according to any one of claims 1-12 and the pharmaceutical composition of claim 13 for use in the treatment and/or prevention of an inflammatory disease.

19. The colloidal particle according to any one of claims 1-12 and the pharmaceutical composition of claim 13 for use in the treatment and/or prevention of rheumatoid arthritis, intraocular inflammation and/or cancer.

20. The colloidal particle according to any one of claims 1-12 and the pharmaceutical composition of claim 13 for use in the treatment and/or prevention of cancer.

21. The colloidal particle according to any one of claims 1-12, the pharmaceutical composition of claim 13, and the cosmetic formulation of claim 15, for topical and/or intra vitriol applications on the eye.

22. The colloidal particle according to any one of claims 1-12, the pharmaceutical composition of claim 13, and the cosmetic formulation of claim 15, for topical and/or subcutaneous applications on the skin.

23. The colloidal particle according to any one of claims 1-12, the pharmaceutical composition of claim 13 and/or the cosmetic formulation of claim 15 for use in photodynamic therapy.

24. The colloidal particle according to any one of claims 1-12 and the pharmaceutical composition of claim 13 for use in photodynamic therapy for rheumatoid arthritis treatment.

25. A method of treatment by photodynamic therapy the method comprising the steps of administrating to an individual in need thereof an effective amount of the colloidal particle according to any one of claims 1 – 12 or of the pharmaceutical composition according to any one of claims13; and exposing cells and/or a tissue of the individual to a photo effective light.

26. A process for preparing colloidal particles according to any one of claims 1-10 comprising the steps of:

- preparing an aqueous solution (a) of a polycation;
- preparing an aqueous solution (b) of a negatively charged, cyclic molecule;
- adding the aqueous solution (a) to the aqueous solution (b) so as to obtain aqueous solution (c) comprising particles according to any one of claim 1-10.

27. The process of claim 26, further comprising the steps of:

- preparing an aqueous solution (d) of a polyanion;
- adding the aqueous solution (d) to an aqueous solution (e) comprising the particles of aqueous solution (c), so as to obtain aqueous solution (f) comprising particles according to any one of claim 11.

28. The process according to claims 18 or 19 comprising the step of removing the particles of solution (c) from said solution (c).

29. The process according to any one of steps 18 to 20, further comprising the steps of fractionating the particles so as to obtain particles of a desired average diameter and size distribution.

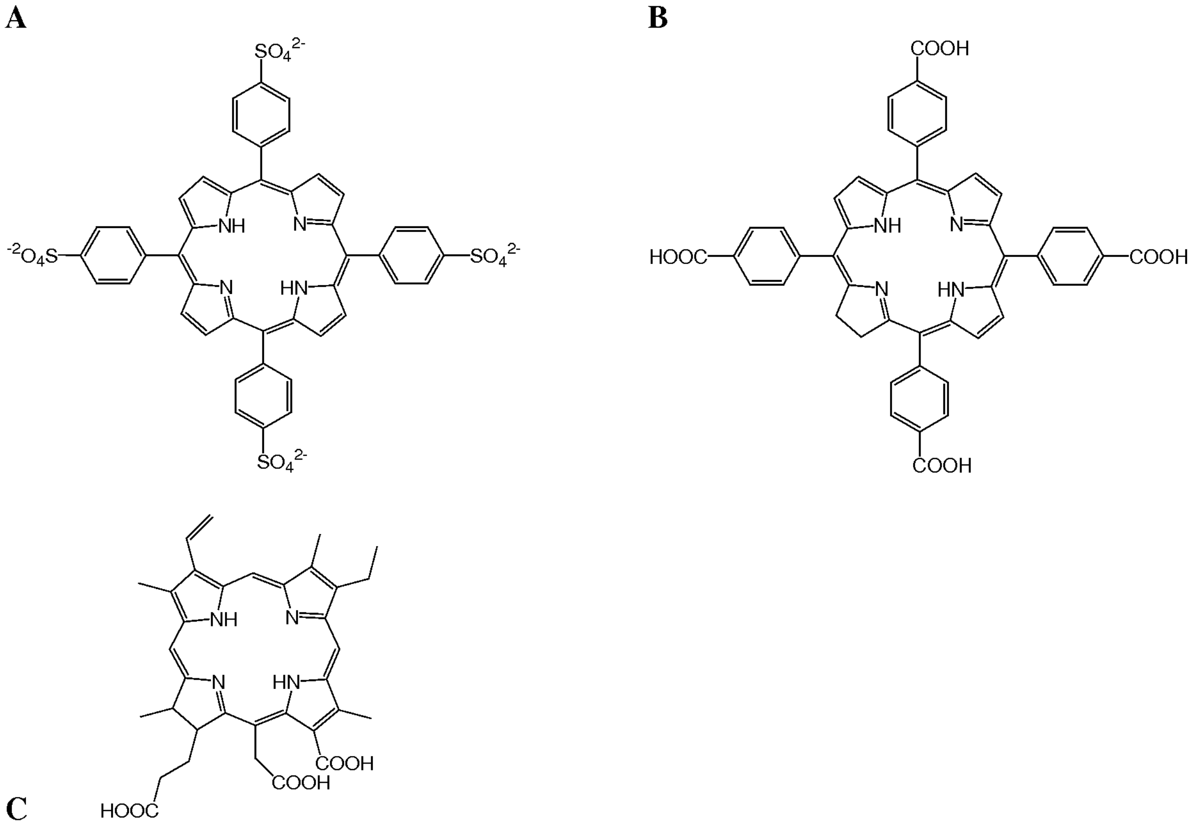

Figure 1

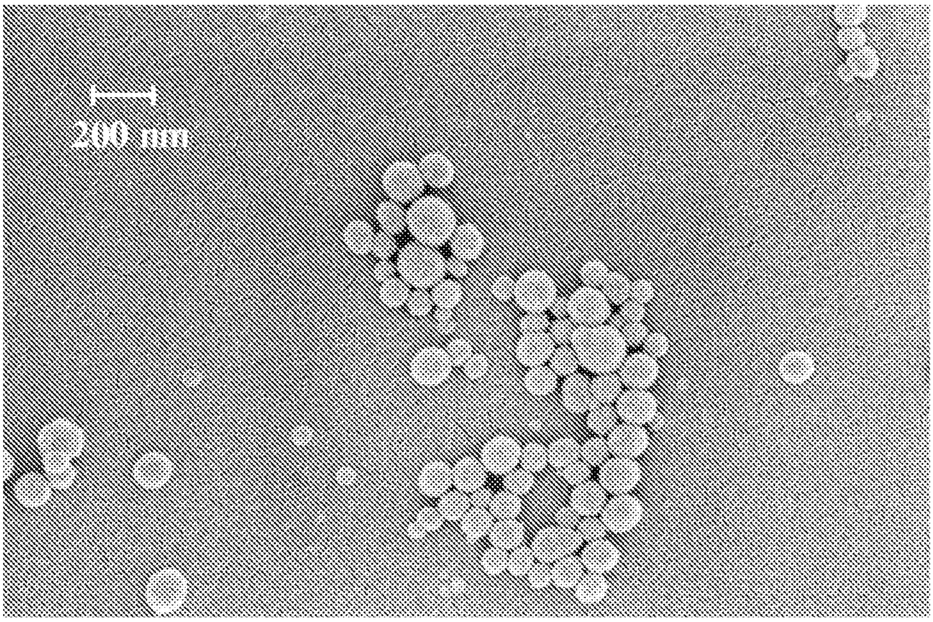

Figure 2

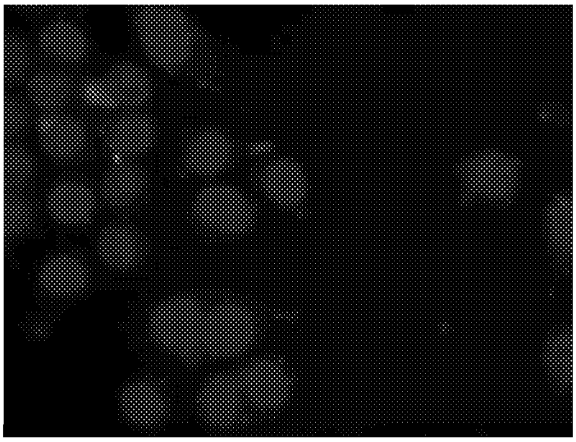

Figure 3 A

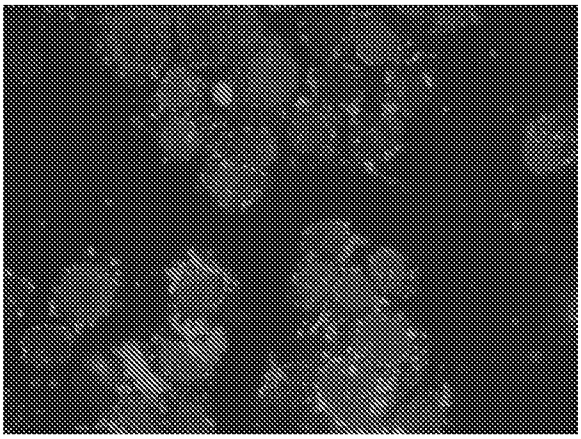

Figure 3 B

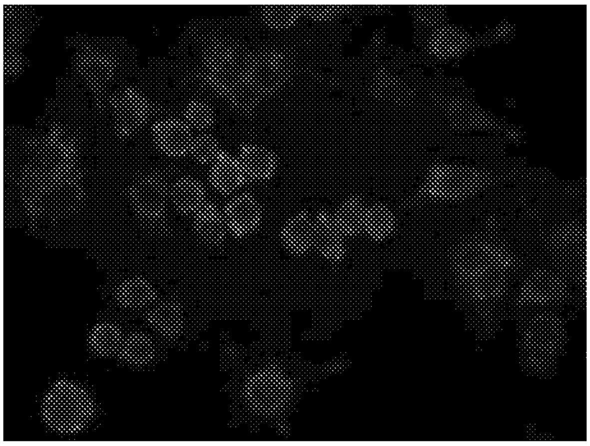

Figure 4 A

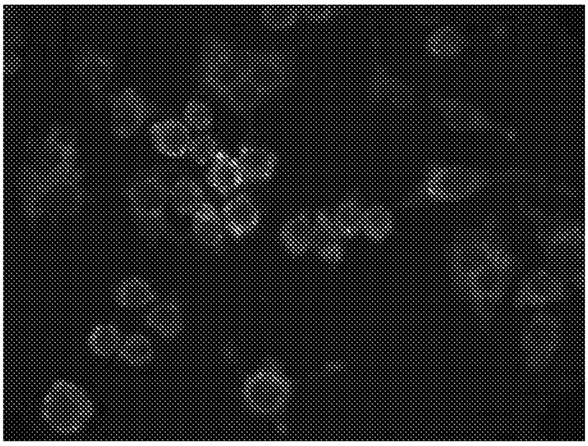

Figure 4 B

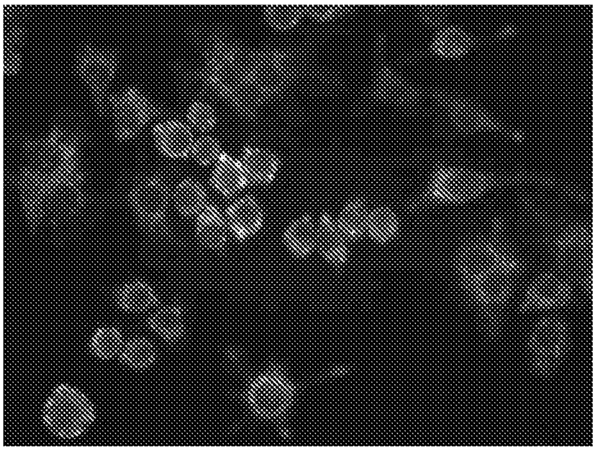

Figure 4 C

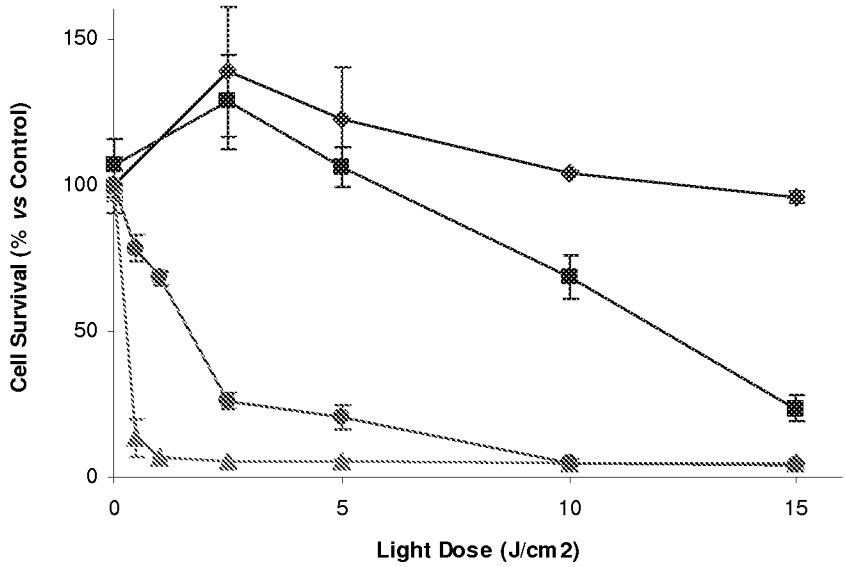

Figure 5

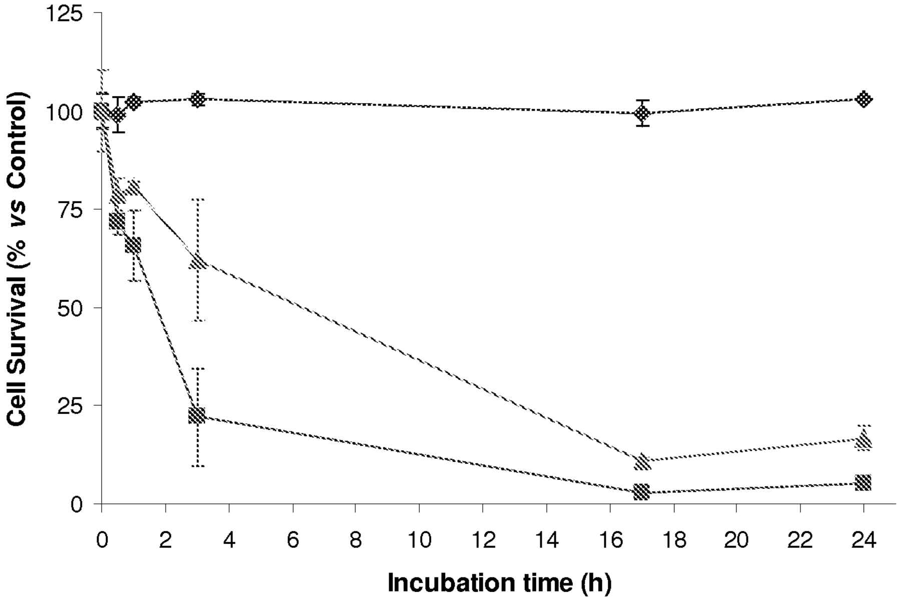

Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2008/055094

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K41/00 A61K49/00 A61P35/00 A61P43/00 A61K47/48
A61K9/51 A61K47/36

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

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

EPO-Internal, BIOSIS, EMBASE, CHEM ABS Data, WPI Data, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 760 467 A (SCHERING AG [DE]) 7 March 2007 (2007-03-07) paragraphs [0041], [0042], [0047], [0048], [0052], [0059], [0069], [0104], [0106] examples 1,5 ----- -/--	1,2, 5-17, 21-23, 25-29

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 2009	14/04/2009

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SYNYTSYA ET AL: "Interaction of meso-tetrakis(4-sulphonatophenyl)porphine with chitosan in aqueous solutions" SPECTROCHIMICA ACTA. PART A: MOLECULAR AND BIOMOLECULAR SPECTROSCOPY, ELSEVIER, AMSTERDAM, NL, vol. 66, no. 2, 4 January 2007 (2007-01-04), pages 225-235, XP005822656 ISSN: 1386-1425 abstract -----	1-6, 8-10,26, 28
X	SOUKOS N S ET AL: "THE EFFECT OF CHARGE ON CELLULAR UPTAKE AND PHOTOTOXICITY OF POLYLYSINE CHLORIN E6 CONJUGATES" PHOTOCHEMISTRY AND PHOTOBIOLOGY, OXFORD, GB, vol. 65, no. 4, 1 January 1997 (1997-01-01), pages 723-729, XP001055298 ISSN: 0031-8655 abstract pages 723-724, section "Material and Methods" -----	1-5, 8-23,25, 26,28
X	HAMBLIN M R ET AL: "IN VIVO FLUORESCENCE IMAGING OF THE TRANSPORT OF CHARGED CHLORIN E6 CONJUGATES IN A RAT ORTHOTOPIC PROSTATE TUMOUR" BRITISH JOURNAL OF CANCER, NATURE PUBLISHING GROUP, LONDON, GB, vol. 81, no. 2, 1 September 1999 (1999-09-01), pages 261-268, XP001146990 ISSN: 0007-0920 abstract -----	1-5, 8-17, 21-23, 25,26,28
X	BISLAND S K ET AL: "Photodynamic Actinometry using Microencapsulates: Concepts and developmental approach" PROCEEDINGS OF SPIE - THE INTERNATIONAL SOCIETY FOR OPTICAL ENGINEERING - APPLICATIONS OF PHOTONIC TECHNOLOGY 6: CLOSING THE GAP BETWEEN THEORY, DEVELOPMENT, AND APPLICATION 2003 SPIE US, vol. 5260, 2003, pages 352-360, XP002521302 abstract -----	1,2,5, 8-17,22, 23,25

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FUCHS B B ET AL: "Susceptibility of Cryptococcus neoformans to photodynamic inactivation is associated with cell wall integrity" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY 200708 US, vol. 51, no. 8, August 2007 (2007-08), pages 2929-2936, XP002521303 ISSN: 0066-4804 abstract page 2935, paragraph 3 -----	1-5, 8-18, 21-23, 25,26,28
P,X	WO 2008/130181 A (KOREA INST SCIENCE TECHNOLOGY [KR]; KWON ICK-CHAN [KR]; CHOI KUI-WON [) 30 October 2008 (2008-10-30) examples 1,6,7 -----	1-9, 13-17, 19-23, 25,26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2008/055094

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 1760467	A	07-03-2007	CA	2619676 A1	08-03-2007
			WO	2007025768 A1	08-03-2007
			US	2007104649 A1	10-05-2007
WO 2008130181	A	30-10-2008	NONE		