(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 416 345 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
08.02.2012 Bulletin 2012/06

(51) Int Cl.:
H01J 49/04 *(2006.01)* ***H01J 49/16*** *(2006.01)*

(21) Application number: **10172180.1**

(22) Date of filing: **06.08.2010**

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR
Designated Extension States:
BA ME RS

(71) Applicants:
- **Philips Intellectual Property & Standards GmbH**
  20099 Hamburg (DE)
  Designated Contracting States:
  DE
- **Koninklijke Philips Electronics N.V.**
  5621 BA Eindhoven (NL)
  Designated Contracting States:
  AL AT BE BG CH CY CZ DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK SM TR

(72) Inventor: **The designation of the inventor has not yet been filed**

(74) Representative: **Jungen, Carmen**
Philips Intellectual Property & Standards GmbH
Weißhausstraße 2
52066 Aachen (DE)

(54) **Particle-based matrix carriers for mass spectrometry**

(57) The present invention relates to a matrix particle comprising a core, and at least one matrix molecule bound to said core, wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a free acid group. The matrix particles may be used in a MALDI process.

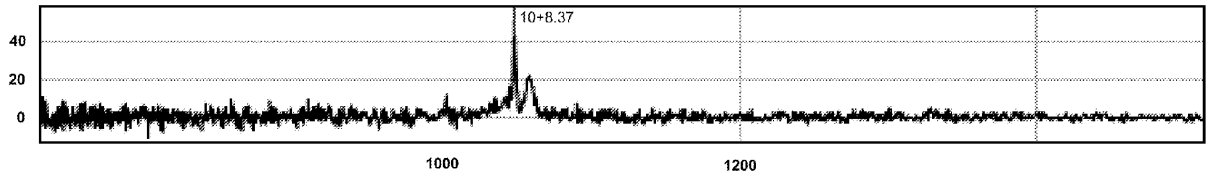

Fig. 4

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to a particle-based matrix carrier for mass spectrometry, especially useful in matrix assisted laser desorption/ionization (MALDI) methods.

BACKGROUND OF THE INVENTION

[0002] Mass spectrometry (MS) is a widely used analytical method for determining the molecular mass of various compounds. It involves transfer of the sample to the gas phase and ionization of the molecules. Molecular ions are separated using electric or magnetic fields in high vacuum based on their mass-to-charge (m/Z) ratios. During the last decades, MS has proven to be an outstanding technique for accurate and sensitive analysis of biopolymers like proteins and peptides. With the introduction of soft ionization techniques such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), it became possible to transfer into the gas phase and ionize these non-volatile, large, and thermally labile molecules without dissociating them.

[0003] In MALDI, the sample is co-crystallized with a so-called matrix, an UV absorbing aromatic compound which is added to the sample in large excess. Common matrices include α-cyano-4-hydroxy cinnamic acid (CHCA) and 3,5-dimethoxy-4-hydroxy cinnamic acid (sinapinic acid, SPA). A pulsed UV laser supplies the energy for ionization and desorption of the sample molecules, the matrix absorbs the UV energy and transfers it to the sample. MALDI ionization is, in most cases, combined with time-of-flight (ToF) analyzers. Separation of ions is achieved by accelerating them in vacuum into a field-free flight tube and measuring their flight time. The flight time of the ions is proportional to their m/Z value. Using MALDI-ToF-MS, molecules with masses over 10^5 Da can be ionized and analyzed without appreciable fragmentation.

[0004] Prior to performing MALDI-MS, complex samples like cell lysates, urine, and blood serum have to be prefractionated in order to eliminate e.g. salts and detergents and to reduce sample complexity. Common prefractionation methods include liquid chromatography, electrophoresis, and isoelectric focusing. WO2008/062372 discloses a device for separating at least one analyte in a liquid sample and further analyzing said analyte by laser desorption/ionization mass spectrometry.

[0005] Both matrix pipetting and co-crystallization of the analyte and the matrix are dependent on temperature and humidity of the surrounding air. Therefore, a large part of the variation in MALDI process relates to the matrix addition step. Moreover, the analysis of low molecular weight biopolymers using MALDI is hindered by the fact, that the matrix itself is also ionized and desorbed. This gives strong background signals at low masses (approximately below 1500 Da), making it almost impossible to detect sample species in this mass range. Also, the reproducibility of the analysis depends on the matrix pipetting an co-crystallization with the analyte, and the coefficients of variation may be low.

[0006] In view of this situation, there is a continuing need for matrix materials and methods reducing the background signals at low masses in MALDI processes. Furthermore, there is also a continuing need for a reproducible MALDI analysis of analytes.

SUMMARY OF THE INVENTION

[0007] It is an object of the present invention to provide a matrix material for MALDI process allowing for a reduced background signal at low masses. It is a further object of the present invention to provide a matrix material allowing for MALDI analysis with a low coefficient of variation.

[0008] The objects of the present invention are solved by the subject-matter of the independent claims, wherein further embodiments are incorporated in the dependent claims.

[0009] It should be noted that the following aspects and preferred embodiments of the invention also apply to the other aspects and embodiments. Especially, if a preferred embodiment is described in relation to, e.g., a first aspect of the invention, it is understood that this embodiment is also applicable to the other aspects of the invention. It is further understood that all embodiments can be freely combined, and do not exclude each other mutually, if not explicitly mentioned.

[0010] An aspect of the invention relates to a matrix particle comprising
a core; and
at least one matrix molecule bound to said core,
wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a free acid group.

[0011] The binding of at least one matrix molecule to said core provides a matrix particle that allows for a matrix material to be used in MALDI analysis resulting in a low background signal at low masses and a high reproducibility.

Furthermore, the use of a matrix molecule bound to a core wherein the matrix molecule further has a free acid group opens the possibility to omit the addition of an acid, like citric acid, to the sample during sample preparation. It is assumed, without being bound to that theory, that the matrix molecule being bound to a core particle is not desorbed, in such a way that it is detected in the MS. With the use of the matrix particles of the present invention, the background signal in mass spectra may be significantly reduced, especially at masses below 2000 Da, or below 1500 Da, or even below 1000 Da.

[0012] Another aspect of the invention relates to a method for the preparation of a particle according to any of the preceding claims, comprising the steps of
providing a core particle; and
chemically or physically binding at least one matrix molecule to the surface of said core particle, wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a carboxylic acid group.

[0013] Still another aspect of the invention relates to the use of a particle according to the first aspect as matrix material in a Matrix Assisted Laser Desorption/Ionization (MALDI) process.

[0014] It can be seen as the gist of the invention that the matrix molecules used in MALDI are fixed to a core particle, and thus the matrix molecules are not detected in the MS. Thus, only analyte molecules will be detected, and the analysis of analyte molecules is not disturbed by matrix molecules. Furthermore, if the core particles are, e.g., fluorescent, it will be possible to determine the amount of matrix particles added to the sample probe in an easy manner.

[0015] These and other aspects and embodiments of the present invention will be described in the following with reference to the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016]

Fig 1 shows the IR spectrum of matrix particles prepared according to Comparative Example 1.
Fig. 2 shows the IR spectrum of matrix particles prepared according to Example 3.
Fig. 3 shows the IR spectrum of matrix particles prepared according to Example 4.
Fig. 4 shows the SELDI-MS spectrum of Angiotensin II using matrix particles of Example 3.
Fig. 5 shows the size distribution of nanoparticles before and after protein functionalization according to Example 7.

DETAILED DESCRIPTION OF EMBODIMENTS

[0017] A first aspect of the invention relates to a matrix particle comprising
a core; and
at least one matrix molecule bound to said core,
wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a free acid group.

[0018] In a preferred embodiment, the matrix particle further comprises at least one shell covering said core. In other words, the core of the matrix particle is coated with a shell material form a core with at least one shell covering said core.

[0019] In a further preferred embodiment, the matrix particle consists of a core, optionally at least one shell covering said core, and a matrix molecule.

[0020] The matrix molecule is bound to said core. In the following, when the matrix molecule is bound to said core, the matrix molecule may be bound directly to the core, i.e. directly to the core material, or, if an additional shell is present covering the core, the matrix may be bound to the shell, i.e. to the shell material. If several shells are covering the core, e.g. in the manner of an onion-like arrangement, the matrix material is bound to the outer most shell. It should be ensured that the matrix molecule is on the outer side of the matrix particle and can be directly irradiated by laser light.

[0021] The binding of the at least one matrix molecule to said core, or core particle, can be a physical or chemical binding. If the matrix molecule is bound physically, the matrix molecule may be adsorbed to the surface of the core particle, e.g., by van der Waals forces, or by ionic forces. On the other hand, if the matrix molecule is chemically bound to the core, a covalent bond is established between the core and the matrix molecule.

[0022] It should be understood that the binding of the matrix molecule can be achieved in different ways and may comprise covalent bonding of the matrix molecule to the core, or a covalent bonding of the matrix molecule to a linker molecule, which in turn may be bound to the core in different ways. It is anticipated that said linker molecule may be directly bound to the core, e.g. by covalent bonding, or attached to the core in another manner, like by ionic bonding, or by adhesion or cohesion forces binding the linker molecule to the core material. Such core particles having a matrix molecule bound to the core particle may also be termed as "matrix-functionalized core particle" or "matrix-functionalized particle".

[0023] In a preferred embodiment of the invention, the matrix molecule is chemically bound to the core, preferably by covalent bonding.

[0024] The matrix molecule used according to the invention carries an acid group. Said acid group is a "free acid group" wherein the acid group has a proton or a negative charge. Said acid group is not used for binding any other groups to the matrix molecule, or for binding the matrix molecule itself to the core.

[0025] In a preferred embodiment, the free acid group is a carboxylic acid group (-COOH), or an acid amide or amide group ($-CONH_2$), with the carboxylic acid group being preferred. It is understood that the molecule may have more than one of these groups, and also combinations of the groups are possible.

[0026] By the term "the molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI)" it is meant that the matrix molecule may absorb light in the given range. The absorption spectrum of the matrix molecule is determined using the free, unbound molecule. It is understood that the absorption maximum of the matrix molecule may change when the molecule is bound to the core.

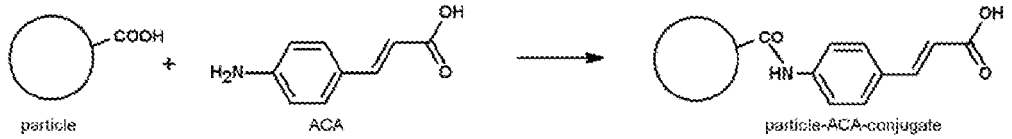
[0027] The matrix molecule is bound to the core or core particle. In this context, the terms "core" and "core particle" are used interchangeable. As outlined above, it is obvious to a person skilled in the art that the core or core particle is intended to also comprise a core particle being coated or covered with a shell, or even several shells, if reference is made to the matrix molecule being bound to the core.

[0028] In another preferred embodiment, the matrix molecule is selected from the group consisting of 4-aminocinnamic acid (ACA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SPA), 2,5-dihydroxybenzoic acid (DHBA), 2-iodoacetamide (IMA), alpha-cyano-4-hydroxy cinnamic acid, SDHB (mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid), trans indole-3-acrylic acid, 2-(4-hydroxyphenylazo)benzoic acid (HABA), succinic acid, ferulic acid, caffeic acid, 3-hydroxypicolinic acid (HPA), picolinic acid (PA), anthranilic acid, nicotinic acid, salicylamide, trans 3-indoleacrylic acid (IAA), and 2,5-dihydroxybenzoic acid (DHB), preferably 4-aminocinnamic acid (ACA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SPA), and 2,5-dihydroxybenzoic acid (DHBA), and derivatives thereof It is further preferred that the matrix molecule is selected from the group consisting of 4-aminocinnamic acid (ACA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SPA), and 2,5-dihydroxybenzoic acid (DHBA), and derivatives thereof. It is understood to a person skilled in the art that also combinations of matrix molecules may be used, namely, two or more different matrix molecules may be used in with a single core.

[0029] By "derivatives thereof" in connection with the matrix molecule, derivatives of the above given matrix molecules are anticipated wherein the matrix molecule is modified with a linker group linking the matrix molecule in a chemical binding to the core. The matrix molecules may be bound or attached to the core via reactive groups present in the matrix molecule, like, e.g., hydroxyl groups (-OH) or amine groups (-NH), as they are, e.g., present in CHCA, SPA, DHBA, or ACA, respectively. Other groups allowing for an attachment of the matrix molecule to the core are, e.g., alkyl halogens ($-(CH_2)n-Cl$) (wherein n is between 0 and 8, preferably between 1 and 4), or isocyanates (-N=C=O). In order to achieve amine-reactivity, the particle surface may be provided with are isothiocyanates (-N=C=S), isocyanates (-N=C=O), acyl azides ($-CO-N2^+$), carboxylates (-COOH), sulfonyl chlorides ($-SO_2Cl$), aldehydes (-CHO), epoxides, carbonates, or anhydrides. If such groups are, e.g., introduced into one of the above given matrix molecules, the matrix molecules resembles a derivative of the matrix molecule being readily adapted for binding to the core.

[0030] In a preferred embodiment, the core or the at least one shell provides groups for binding or attaching a matrix molecule to the core, or to the at least one shell, respectively. In a further preferred embodiment, the core or the at least one shell provides amine groups ($-NH_2$), hydroxyl groups (-OH), boronic acid groups ($-B(OH)_2$), aldehyde groups (-CHO), phosphate groups ($-PO_4^{3-}$), thiol groups (-SH), and/or carboxylic acid groups (-COOH) at the surface as possible binding cites for binding matrix molecules and/or for binding target molecules.

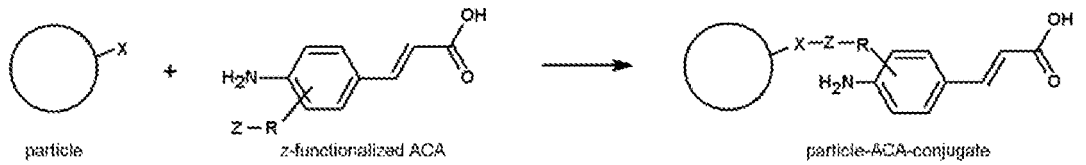
[0031] As an exemplary embodiment, the binding of ACA to a core via a carboxylic acid group on the core and an amine group at the ACA is depicted in Scheme 1 below, and is experimentally described in Example 6 below:



Scheme 1: Coupling of matrix molecule ACA to core particle.

[0032] It is further possible that the matrix molecule is transferred into a derivative providing a reactive group for binding to the core, if such a group is not present in the matrix molecule, or should not be used for binding. The reactive group, depicted as Z in Scheme 2 below, may further be attached to a spacer, depicted as R in Scheme 2 below. Spacer R may be, e.g., an alkanediyl, preferably $-(CH_2)_m-$, wherein m is in the range of 1 to 8, preferably in the range of 2 to 4.

Group Z in Scheme 2 below may be any of the reactive groups given above, and group X in Scheme 2 below may be any group provided on the surface of the core, as outlined above. It is obvious to a person skilled in the art that groups X and Z in Scheme 2 below might be modified, as required by a chemical reaction, depending on the nature of X, Z and the chemical reaction, and might thus be modified groups X and Z, respectively, or might not at all be present any more.

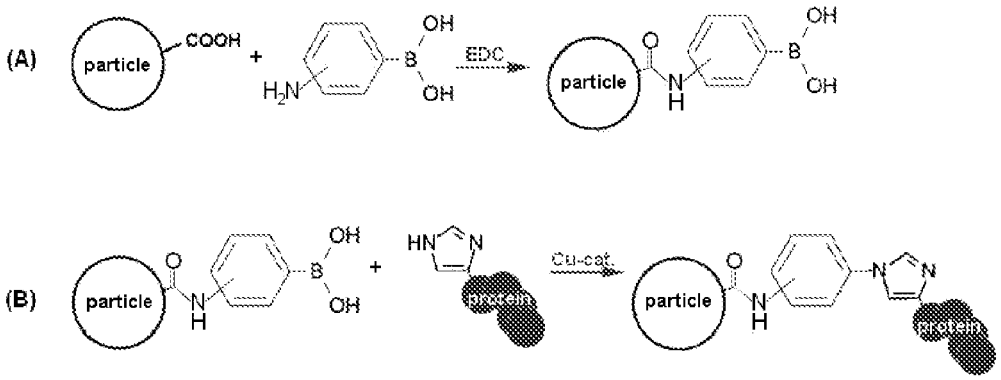


Scheme 2: Coupling of matrix molecule ACA via a linker group R and a reactive group Z to particle.

[0033] The matrix-functionalized particles may additionally be provided with targeting units like biomolecules (peptide, protein, polynucleotides, etc) recognizing specific target moieties of a sample. The conjugation of biomolecules to the particles can be performed in the same manner as done for the matrix compounds. The same reactive groups onto the particle surface can be used for the conjugation chemistry (see above). Omnipresent reactive residues on the biomolecules like carboxylates (-COOH), amines ($-NH_2$), thiols (-SH), phosphates ($-PO_4^{3-}$), and hydroxyls (-OH) may be used for binding.

[0034] In an exemplary embodiment, the reaction with COOH-functionalized luminescent particles and a protein (anti-CEA scFv) via the N-terminal site (comprising a primary amine group, R $-NH_2$) can experimentally be performed as shown in Example 7.

[0035] In another exemplary embodiment, a more site-specific reaction between a matrix-functionalized particle and a histidine-containing biomolecule (such as peptides or proteins) may be performed as claimed in WO2006064451, which is hereby explicitly incorporated by reference. The Cu-catalyzed reaction results in a covalent binding between an arylboronic acid derivate and imidazole as present in the amino acid histidine (His). Histidine is very often applied in recombinant proteins for purification reasons as so-called His-tag at Nor C-terminal sites. A His-specific reaction may be a conjugation method to retain the physiological properties of proteins such as antibodies. The His-specific conjugation reaction may be performed in two steps. Firstly, the particles may be equipped with arylboronic acid units using an EDC-mediated reaction. This may be applied with COOH-functionalized particles and a primary amine-functionalized arylboronic acid derivate as described in Scheme 3(A). In the second step, the arylboronic-acid-functionalized particles can react with the imidazole residue of a histidine-containing protein by means of a Cu-catalyst reaction (Scheme 3(B)). A detailed exemplary reaction procedure is given as Example 8.



Scheme 3: (A) EDC-mediated reaction to functionalize particles with arylboronic acid derivates. (B) Cu-catalysed reaction to conjugate proteins in a His-specific manner.

[0036] In another preferred embodiment, the core comprises, or consists of, an organic, an inorganic material, or a

mixture thereof.

[0037] In a further preferred embodiment, the inorganic material of the core is selected the group consisting of optionally doped oxides, optionally doped carbonates, optionally doped fluorides, optionally doped aluminates, optionally doped germanates, optionally doped titanates, optionally doped vanadates, optionally doped niobates, optionally doped tantalates, optionally doped molydates, optionally doped tungstates, optionally doped phosphates, metals, inorganic semiconductors, and combinations thereof.

[0038] Examples of such inorganic core materials are MeO, Ln_2O3, SiO_2, TiO_2, ZrO_2, HfO_2, ZnS, $LnPO_4$, $LnBO_3$, LnB_3O_6, $LnMgB_5O_{10}$, $MeCO_3$, $Ln_2(CO_3)_2$, $MeAl_2O_4$, $MeMgAl_{10}O_{17}$, $LnMgAl_{11}O_{19}$, Al_2O_3, $Me_2Al_2O_5$, $LnAlO_3$, $MeAl_4O_7$, $Ln_4Al_2O_9$, $A_2Al_2O_4$, $Ln_3Al_5O_{12}$, $Ln_3Ga_5O_{12}$, $Mg_4(Si,Ge)O_{5\cdot5}F$, Mg_2TiO_4, $Ln_2(Ti,Zr,Hf)_2O_7$, $Me_2Ln_2TiO_7$, $MeTiO_3$, $Ln(V,P,Nb,Ta)O_4$, $Ln_2(Mo,W)_3O_{12}$, $ALn(Mo,W)_2O_8$, iron oxide (FeO, Fe_2O_3, Fe_3O_4), $LnPO_4$, gold (Au), silver (Ag), cadmium selenide (CdSe), cadmium sulfide (CdS), cadmium telluride (CdTe), indium phosphide (InP), wherein
Me is selected from Mg, Ca, Sr, Ba, Zn;
Ln is selected from Sc, Y, La, Gd, Lu, In; and

[0039] A is selected from Li, Na, K, Rb, Cs. and whereas the dopand is selected from a group comprising Ti^{2+}, V^{3+}, C^{4+},Mn^{5+}, Tf^{+}, V^{2+}, C^{3+}, Mn^{4+}, Fe^{3+}, Ru^{3+}, Co^{3+}, Co^{2+}, Ir^{3+}, Ni^{2+} ,Pt^{2+} ,Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Dy^{3+}, Er^{3+}, Tm^{2+}, Tm^{3+}, Yb^{3+},Yb^{2+},In^{+}, Sn^{2+}, Pb^{2+} ,Bi^{3+}.

[0040] Especially preferred inorganic core materials are selected from the group consisting of silica (SiO_2), cadmium selenide (CdSe), cadmium sulfide (CdS), cadmium telluride (CdTe), indium phosphide (InP), gold (Au), iron oxide (FeO, Fe_2O_3, Fe_3O_4), and rare-earth doped yttrium phosphate (YPO_4) or lanthanum phosphate ($LaPO_4$), and combinations thereof. In a specifically preferred embodiment, the inorganic material is europium (Eu) doped yttrium phosphate or lanthanum phosphate.

[0041] Whenever materials are "optionally doped", these materials can be used as pure materials, or doped with another element. In a preferred embodiment, the dopant is selected from the group consisting Ti^{2+},V^{3+}, Cr^{4+}, Mn^{5+}, Ti^{+}, V^{2+}, Cr^{3+}, Mn^{4+}, Fe^{3+}, Ru^{3+}, Co^{3+}, Co^{2+}, Ir^{3+},Ni^{2+}, Pt^{2+}, Pr^{3+}, Nd^{3+}, Sm^{3+},Eu^{3+}, Dy^{3+}, Er^{3+}, Tm^{2+}, Tm^{2+}, Tm^{3+}, Yb^{3+}, Yb^{2+}, In^{+}, Sn^{2+}, Pb^{2+}, Bi^{3+}. In another preferred embodiment, the dopant is a rare-earth element. The amount of doping, preferably in the rare-earth doped materials, and especially in rare-earth doped yttrium phosphate or lanthanum phosphate, may be at or below 30 % by weight, or at or below 20 % by weight, or even at or below 10 % by weight.

[0042] In still another preferred embodiment, the organic material of the core is selected the group consisting of polyacrylic acid and derivatives thereof, polymaleic acid and derivatives thereof, polyethyleneglycol (PEG), phospholipids, block copolymers, poly(α-cyano-4-hydroxy cinnamic acid), and poly(sinapinic acid). It is also anticipated by the present invention that matrix material, like, e.g., α-cyano-4-hydroxy cinnamic acid or sinapinic acid, is polymerized and used as a core material. Thus, the matrix material is present in the core, and also at the same time on the surface of said core, and a specific functionalization is not necessary any more.

[0043] In another preferred embodiment, the core and/or the at least one shell is luminescent, preferably photoluminescent, further preferably phosphorescent or fluorescent, and/or magnetic. The use of a luminescent particle as matrix particle for MALDI processes allows for a determination of the amount of particle, and thus the amount of matrix, added to the sample probe in an easy way.

[0044] The luminescence of the core may, e.g., be achieved by doping the core with rare earth elements, as described above, or in another preferred embodiment, by phosphorescent or fluorescent dyes. In a more preferred embodiment, the organic material of the core is further doped with at least one dye, preferably a fluorescent dye, more preferably selected from the group consisting of cumarin, luciferin, fluorescein, rhodamines, carbazines, safranines, oxazines, thiazines.

[0045] As outlined above, the core may be coated with at least one shell. Each of these shells (which may comprise a monolayer or a polylayer of an appropriate material in preferred embodiments of the present invention) has a thickness of about 0.5 nm to 100 nm. In a preferred embodiment of the present invention, each shell has a thickness of about 0.5 nm to 500 nm and can be made of different materials or of the same material. Furthermore, the shell can cover the core at least partially.

[0046] In a still further preferred embodiment, the shell may comprise an inorganic or organic shell, preferably a material selected from the group consisting of gold, $Si0_2$, ZnS, a polyphosphate (e.g. calcium polyphosphate), an amino acid (e.g. cysteine), an organic polymer (e.g. polyethyleneglycol/PEG, polyvinylalcohol/PVA, polyamide, polyacrylate, polyurea), a biopolymer (e.g. a polysaccharide, like, e.g., dextran, xylan, glycogen, pectin, cellulose, or polypeptide like collagen, globulin), cysteine or a peptide with high cysteine content, or a phospholipid. Examples of organic shell materials may comprise, e.g. (not limited to these), carboxylic acids, acid halides, amines, acid anhydrides, activated esters, maleimides, isothiocyanates, a polyphosphate (e.g. calcium polyphosphate), an amino acid (e.g. cysteine), an organic polymer (e.g. polyethylene glycol/ PEG, polyvinyl alcohol/PVA, polyamide, polyacrylate, polyurea), an organic functional polymer (e.g. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)2000] ammonium salt), a biopolymer (e.g. polysaccharide such as dextran, xylan, glycogen, pectin, cellulose or polypeptide such as collagen, globulin), cysteine or a peptide with a high cysteine content or a phospholipid. A very preferred embodiment relates to

the combination of a core of CdSe or CdS and a shell of ZnS.

[0047] In a preferred embodiment, the core or at least one shell, preferably the outermost shell, provide amine groups ($-NH_2$), hydroxyl groups (-OH), boronic acid groups ($-B(OH)_2$), aldehyde groups (-CHO), phosphate groups ($-PO_4^{3-}$), thiol groups (-SH), and/or carboxylic acid groups (-COOH) at the surface as possible binding cites for binding matrix molecules and/or for binding target molecules. These groups may be used for further functionalization of the core particles with, e.g., matrix molecules or target molecules.

[0048] In another preferred embodiment, the core particles may have a size in the range of from 1 to 10 μm, preferably in the range of from 5 to 1 μm, further preferably in the range of from 10 to 500 nm, and most preferably in the range of 100 to 200 nm. The size of the core particles may relate to the core itself, without any shells, or to the entire core particle including any optionally applied shell(s).

[0049] Another aspect of the present invention relates to a method for the preparation of a matrix particle according to the first aspect of the invention, comprising the steps of providing a core particle; and

[0050] chemically or physically binding at least one matrix molecule to the surface of said core particle, wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a carboxylic acid group.

[0051] It is obvious to a person skilled in the art that any of the above given embodiments described in relation to the matrix particle also applies to the method for the preparation of the matrix particle. This specifically applies to the reactions, e.g., for the functionalization of the particle, given above, and also to the application of a shell prior to the functionalization.

[0052] A third aspect of the present invention relates to the use of the matrix particles in a Matrix Assisted Laser Desorption/Ionization (MALDI) process or experiment.

[0053] As also outlined above, MALDI is a powerful tool in the characterization of molecules, and especially proteins. The present invention provides matrix particles which can be used as matrix material in MALDI, or also in SELDI (Surface Enhanced Laser Desorption/Ionization) and SEND (Surface Enhanced Neat Desorption).

[0054] In a preferred embodiment, whenever reference is made to MALDI, SELDI or SEND, the light used may be in the range of from 100 nm to 100 μm, preferably from 200 nm to 1000 nm, or preferably from 1 μm to 10 μm, further preferably from 250 to 600 nm, further preferably in the range of 325 to 350 nm, and most preferably about at 337 nm or at about 532 nm.

[0055] The matrix particles of the present invention may be used similar to classical matrix materials, like matrix molecules. In a similar manner, the matrix particles may be applied as a suspension of the matrix particles in conventionally known solvents, like, e.g., ethanol, 0.5 % trifluoric acid and 50 % acetonitrile in water, or in 0.1 % trifluoric acid in water, or any other combination of these solvents.

[0056] In another preferred embodiment, the matrix particles of the present invention are used as matrix material in MALDI, SELDI or SEND without the addition or use of an additional acid, like, e.g., citric acid, and/or glycerol. Thus, the final sample probe does not contain any additional acid not part of the matrix particles of the present invention, and/or glycerol.

[0057] Once the matrix particles are applied to the sample probe, the amount of matrix particles may be determined by luminescence, preferably photoluminescence. This is advantageous over the conventional methods. The amount of matrix added to a sample probe is depending on the concentration of the solution of the matrix molecule and the small amount of liquid added. However, the variations in concentration and amount of the solution are substantive. The matrix particles of the invention now allow for a measuring of the amount of matrix particles added by luminescence. Thus, the reproducibility of MALDI processes may be increased.

[0058] In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality.

[0059] The present invention is now further described by the following Examples, which are not understood to be limiting, but rather exemplarily. It is further noted that the above given embodiments may be combined in any way.

EXAMPLES

Comparative Example 1

[0060] Synthesis of Eu-containing yttrium phosphate nanoparticles in suspension

[0061] Europium-containg yttrium phosphate nanoparticles were synthesized and characterized as described below.

[0062] Three solutions A, B and C were prepared as follows:

Solution A: 1.795 g (6.5 mmol) $Y(CH_3COO)_3.H_2O$ and 0.237 g (0.72 mmol, 10 mol%) $Eu(CH_3COO)_3 \cdot H_2O$ were dissolved in 20 ml H_2O, and then 20 drops pure acetic acid were added. A transparent solution was obtained.
Solution B: 10 ml ethanol.
Solution C: 0.828 g (7.2 mmol) $NH_4H_2PO_4$ were dissolved in 5 ml H_2O by stirring at room temperature. A clear

solution at pH 4.08 was obtained. Then, NH_4OH (conc.) was added to increase the pH to pH 9.57.
Solution B was added to Solution A under stirring at room temperature. A clear, transparent solution was obtained. Then, Solution C was added very quickly. A fine white suspension was generated which was treated with ultrasonic sound for 15 min. The pH was increased to pH 6.8. The neutral suspension was stored at room temperature in darkness for 16 hours. The resulting IR spectrum is shown in Fig. 1.

Example 2

[0063] Synthesis of Eu-containing yttrium phosphate nanoparticles coated with SPA

[0064] Europium-containg yttrium phosphate nanoparticles were synthesized, coated with SPA, and characterized as described below.

[0065] Solution A: 1.795 g (6.5 mmol) $Y(CH_3COO)_3 \cdot H_2O$ and 0.237 g (0.72 mmol, 10 mol%) $Eu(CH_3COO)_3 \cdot H_2O$were dissolved in 20 ml H_2O,and then 20 drops pure acetic acid were added. A transparent solution was obtained.

[0066] Solution B: 0.265 g (1.18 mmol, 15.8 mol%) 3,5-dimethoxy-4-hydroxy cinnamic acid (Sinapinic acid, SPA) were dissolved in 10 ml ethanol. A clear, transparent, yellowish solution was obtained.

[0067] Solution C: 0.828 g (7.2 mmol) $NH_4H_2PO_4$ were dissolved in 10 ml $H_2O$by stirring at room temperature. A clear solution was obtained. Then, NH_4OH (conc.) was added to increase the pH to pH 9.85.

[0068] Solution A was treated in an ultrasonic bath. Solution B was very quickly completely added during treatment in the ultrasonic bath. A clear, transparent solution was obtained. Then, Solution C was very quickly added, and a white suspension was generated. The ultrasonic treatment was continued for further 15 min.

[0069] After 16 hours storage at room temperature, a light orange, stable suspension with no sedimentation of solid was obtained. The suspension was centrifuged (5000 rpm, 15 min), the light orange, clear, transparent solution was decanted, and the remaining solid was twice washed with water, centrifuged and the supernatant was decanted. A light orange solid was obtained.

Example 3

[0070] Synthesis of Eu-containing yttrium phosphate nanoparticles coated with SPA

[0071] Europium-containg yttrium phosphate nanoparticles were synthesized, coated with SPA, and characterized as described below.

[0072] Solution A: 1.795 g (6.5 mmol) $Y(CH_3COO)_3 \cdot H_2O$and 0.237 g (0.72 mmol, 10 mol%) $Eu(CH_3COO)_3.H_2O$ were dissolved in 20 ml H_2O, and then 20 drops pure acetic acid were added. A transparent solution was obtained.

[0073] Solution B: 0.265 g (1.18 mmol, 15.8 mol%) 3,5-dimethoxy-4-hydroxy cinnamic acid (Sinapinic acid, SPA) were dissolved in 10 ml ethanol. A clear, transparent, yellowish solution was obtained.

[0074] Solution C: 0.828 g (7.2 mmol) $NH_4H_2PO_4$ were dissolved in 10 ml H_2O by stirring at room temperature. A clear solution was obtained. Then, NH_4OH (conc.) was added to increase the pH to pH 9.85.

[0075] Solution B was added to Solution A under stirring at room temperature, resulting in a clear, transparent yellowish solution. After 10 minutes, suspension having pH 4.41 was obtained. Pure acetic acid was added to decrease the pH to 2.58, resulting in a clear, transparent solution. After 15 min of treatment in an ultrasonic bath, Solution C was very quickly added. A fine, yellowish suspension was generated, which was further treated in an ultrasonic bath for 15 min.

[0076] The pH was then increased from 5.24 to 6.85, and the neutral suspension was stored at room temperature in darkness for 16 hours. The suspension was centrifuged (5000 rpm, 15 min), the supernatant was decanted, and the remaining solid was twice washed with water, centrifuged and the supernatant was decanted. The IR spectrum is shown in Fig. 2.

Example 4

[0077] Synthesis of Eu-containing yttrium phosphate nanoparticles coated with CHCA

[0078] Europium-containg yttrium phosphate nanoparticles were synthesized, coated with CHCA, and characterized as described below.

[0079] Solution A: 1.795 g (6.5 mmol) $Y(CH_3COO)_3 \cdot H_2O$ and 0.237 g (0.72 mmol, 10 mol%) $Eu(CH3COO)_3 \cdot H_2O$ were dissolved in 20 ml H_2O, and then 20 drops pure acetic acid were added. A transparent solution at pH 2.9 was obtained.

[0080] Solution B: 0.265 g (1.18 mmol, 15.8 mol%) α-cyano-4-hydroxy cinnamic acid (CHCA) were dissolved in 10 ml ethanol. A clear, transparent, yellowish solution was obtained.

[0081] Solution C: 0.828 g (7.2 mmol) $NH_4H_2PO_4$ were dissolved in 5 ml H_2O by stirring at room temperature. A clear solution was obtained. Then, NH_4OH (conc.) was added to increase the pH to pH 9.63.

[0082] Solution A was treated in an ultrasonic bath. Solution B was very quickly completely added during treatment in the ultrasonic bath. A clear, transparent yellow solution at pH 4.36 was obtained. The solution was further treated in

the ultrasonic bath for 15 min.

[0083] Then, Solution C was very quickly added, and a yellow suspension was generated. The ultrasonic treatment was continued for further 15 min. The resulting suspension is at pH 5.18. NH_4OH was added to increase the pH to 6.71. The suspension was stored at room temperature in darkness for 16 hours.

[0084] The light green suspension with no sedimentation of solid was centrifuged (5000 rpm, 15 min), the supernatant was decanted, and the remaining solid was three times washed with water, centrifuged and the supernatant was decanted. The supernatant after washing was green after all three washes. The resulting solid was dried at 120 °C and characterized by IR; the IR spectrum is shown in Fig. 3.

Example 5

[0085] Mass spectrometric testing of synthesized matrix-carrying nanoparticles

[0086] The particles synthesized according to the above Examples were tested using the PS 4000 Enterprise Edition SELDI-MS system (Bio-Rad Laboratories) in order to test their inherent matrix activity. Normal phase SELDI chips (NP20, Bio-Rad Laboratories) were used as targets.

[0087] The powders of Comparative Example 1, Example 3, and Example 4 were compared in the same manner. The powders of all three Examples were each suspended in ethanol, in 0.5 % trifluoric acid and 50 % acetonitrile in water, and in 0.1 % trifluoric acid in water, resulting in nine different suspensions.

[0088] The powders were dispersed in the respective solvents using ultrasonic bath mixing of 0.02g powder in 800 µl solvent for 5min. The peptide standard Angiotensin II with m=1045.5Da (ProteoMass MALDI-MS Standard from Sigma-Aldrich) was used as a model analyte. Prior to MS experiments, the NP20 surface was washed three times with 4µl water and air dried. Subsequently, 5µl Angiotensin II solution (100 pmol/µl, dissolved in water) was added to the surface and dried. After this, 2µl matrix particle suspension was added and allowed to air dry on the spot. Finally, 2µl more Angiotensin II solution was added to each spot.

[0089] The spots were analyzed by SELDI-MS with and without matrix particle addition. The results illustrate that the matrix particles indeed exhibit inherent MALDI matrix activity. The spectra were acquired under the same experimental conditions (Laser intensity: 7000, Focus mass: 1050 Da, Matrix deflection: 500 Da). It was found that the matrix particles of Example 3 dispersed in 0.1 % TFA worked the best as matrix, see Fig. 4 showing the mass of Angiotensin II (1045.5 Da), with m/z on the x-axis, and the intensity in counts on the y-axis.

Example 6

[0090] The conjugation with COOH-functionalized particles and ACA can experimentally be done as follows:

55 µl 10x PBS solution (PBS = phosphate buffer saline: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4)
100 µl particle solution (9.2 µM)
184 µl 10 mM EDC solution (EDC = 1-ethyl-3-dimethylaminopropyl) carbodiimide hydrochloride)
184 µl 10mM sulfo-NHS solution (N-hydroxysulfosuccinimide sodium salt) Incubation at room temperature (30 min)
0.5 µl 2-mercaptoethanol
Mixing for 15 min
18 µl 10 mM p-aminocinnamic acid
Mixing at room temperature (2 h)
Separation of unbound ACA by ultrafiltration spin filter devices

Example 7

[0091] The reaction with COOH-functionalized luminescent particles and a protein (anti-CEA scFv) via the N-terminal site (comprising a primary amine group, R -NH_2) can experimentally be performed as follows:

35.6 µl PBS solution (PBS = phosphate buffer saline: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4)
15 µl COOH-functionalized CdSe/ZnS nanoparticles solution (8 µM)
60.4 µl anti-CEA scFv antibody fragment solution
9 µl 20 mM EDC solution (EDC = 1-ethyl-3- dimethylaminopropyl carbodiimide hydrochloride)
Mixing at room temperature (2 h)
Separation of unbound peptide molecules by ultrafiltration spin filter devices

[0092] The size distribution of the nanoparticles can be seen from Fig. 5. The left curve at a smaller size shows the size distribution of the nanoparticles before the coupling of the protein as hydrodynamic diameter (measured by dynamic light scattering) with a maximum slightly smaller than the maximum diameter of the protein-functionalized nanoparticles (right curve).

Example 8

[0093] A His-specific reaction with arylboronic acid functionalized particles can experimentally be performed as follows:

As a first step: Arylboronic acid functionalization of particles (CdSe/ZnS nanoparticles)

55 μl water
40 μl 10x PBS solution (PBS = phosphat buffered saline: 10 mM phosphate buffer, 2.7 mM potassium chloride, 137 mM sodium chloride, pH 7,4)
100 μl 0.1 M EDC solution (EDC = 1-ethyl-3- dimethylaminopropyl) carbodiimide hydrochloride)
5 μl 20 mM sulfo-NHS solution (N-hydroxysulfosuccinimide sodium salt)
200 μl 2 μM CdSe/ZnS nanoparticle suspension
Incubation at room temperature (30 min)
10 μl 2-mercaptoethanol
mixing for 15 min
50 μl 20 mM 3-aminophenylboronic acid solution
mixing at room temperature (2 h)
separation of nanoparticles by centrifugation

[0094] As a second step: His-specific conjugation of a fluorescent labeled polyhistidine-oligopeptide to arylboronic acid functionalized CdSe/ZnS nanoparticles
1.7 ml PBS (pH 7.4)
100 μl arylboronic-acid-functionalized CdSe/ZnS nanoparticles (see above)
17 μl FITC-Ahxis6 (FITC=fluoresceinisothiocyanat; Ahx = 6-aminohexene carbonic acid)
100 μl $[Cu(OH)TMEDA]_2Cl_2$
Mixing overnight
[0095] Purification by ultrafiltration and dialysis to remove unbound oligopeptide molecules.

Claims

1. Matrix particle comprising
a core; and
at least one matrix molecule bound to said core,
wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a free acid group.

2. Particle of claim 1, wherein the matrix molecule is selected from the group consisting of 4-aminocinnamic acid (ACA), α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SPA), 2,5-dihydroxybenzoic acid (DHBA), 2-iodoacetamide (IMA), alpha-cyano-4-hydroxy cinnamic acid, SDHB (mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicylic acid), trans indole-3-acrylic acid, 2-(4-hydroxyphenylazo)benzoic acid (HABA), succinic acid, ferulic acid, caffeic acid, 3-hydroxypicolinic acid (HPA), picolinic acid (PA), anthranilic acid, nicotinic acid, salicylamide, trans 3-indoleacrylic acid (IAA), and 2,5-dihydroxybenzoic acid (DHB), preferably 4-aminocinnamic acid (ACA), oc-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SPA), and 2,5-dihydroxybenzoic acid (DHBA), and derivatives thereof.

3. Particle of claim 1, wherein the particle further comprises at least one shell covering said core.

4. Particle of any of the preceding claims, wherein the light used for MALDI is in the range of from 100 nm to 100 μm, preferably from 200 nm to 1000 nm, or preferably from 1 μm to 10 μm, further preferably from 250 to 600 nm, further preferably in the range of 325 to 350 nm, and most preferably about at 337 nm or at about 532 nm.

5. Particle of any of the preceding claims, wherein the particle has a size in the range of from 1 to 10 μm, preferably in the range of from 5 to 1 μm, further preferably in the range of from 10 to 500 nm, and most preferably in the range

of 100 to 200 nm.

6. Particle of any of the preceding claims, wherein said core and/or said at least one shell is luminescent, preferably photoluminescent, further preferably phosphorescent or fluorescent, and/or magnetic.

7. Particle of any of the preceding claims, wherein the core comprises an organic material, an inorganic material, or a mixture thereof.

8. Particle of claim 7, wherein the inorganic material is selected the group consisting of optionally doped oxides, optionally doped carbonates, optionally doped fluorides, optionally doped aluminates, optionally doped germanates, optionally doped titanates, optionally doped vanadates, optionally doped niobates, optionally doped tantalates, optionally doped molydates, optionally doped tungstates, optionally doped phosphates, metals, inorganic semiconductors, and combinations thereof

9. Particle of claim 7, wherein the organic material is selected the group consisting of polyacrylic acid and derivatives thereof, polymaleic acid and derivatives thereof, polyethyleneglycol (PEG), phospholipids, block copolymers, poly (α-cyano-4-hydroxy cinnamic acid), and poly(sinapinic acid).

10. Particle of claim 9, wherein the organic material is further doped with at least one dye, preferably a fluorescent dye, more preferably selected from the group consisting of cumarin, luciferin, fluorescein, rhodamines, carbazines, safranines, oxazines, thiazines.

11. Particle of any of the preceding claims, wherein said shell comprises an inorganic or organic shell, preferably a material selected from the group consisting of gold, SiO_2, ZnS, a polyphosphate, an amino acid, an organic polymer, a biopolymer, cysteine or a peptide with high cysteine content, or a phospholipid.

12. Particle of any of the preceding claims, wherein said core or said at least one shell provide amine groups ($-NH_2$), hydroxyl groups (-OH), boronic acid groups ($-B(OH)_2$), aldehyde groups (-CHO), phosphate groups ($-PO)_4^{3-}$), thiol groups (-SH), and/or carboxylic acid groups (-COOH) at the surface as possible binding cites for binding matrix molecules and/or for binding target molecules.

13. Method for the preparation of a particle according to any of the preceding claims, comprising the steps of
providing a core particle; and
chemically or physically binding at least one matrix molecule to the surface of said core particle, wherein said matrix molecule absorbs light in the range used for Matrix Assisted Laser Desorption/Ionization (MALDI), and said matrix molecule further has a carboxylic acid group.

14. Method of claim 13, wherein said core particle is coated with a shell prior to binding said at least one matrix molecule.

15. Use of a particle according to any of the claims 1 to 12 as matrix material in a Matrix Assisted Laser Desorption/Ionization (MALDI) process.

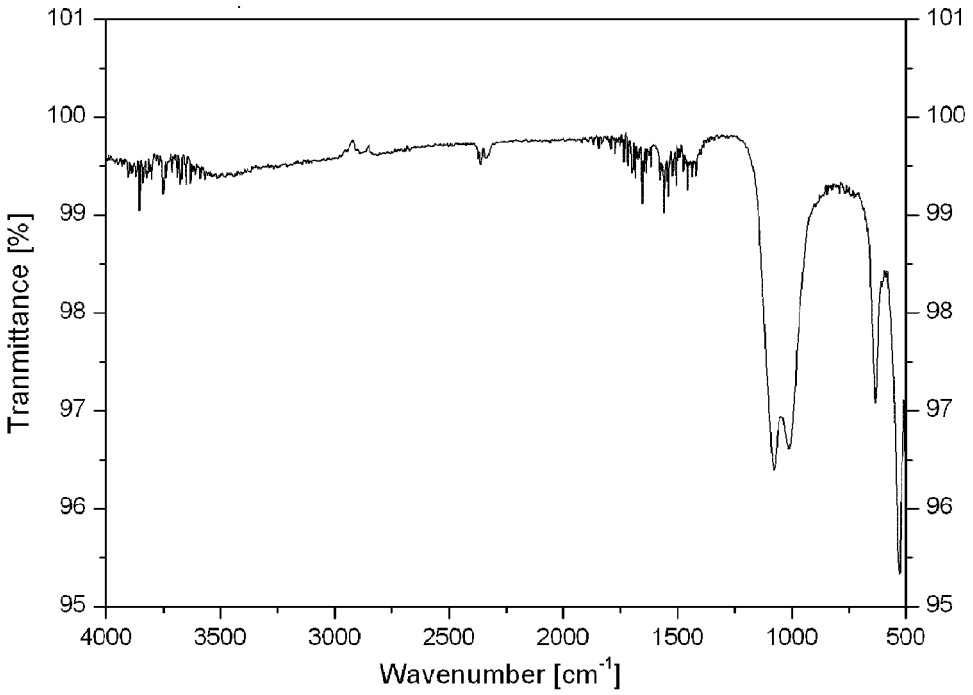

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Fig. 1

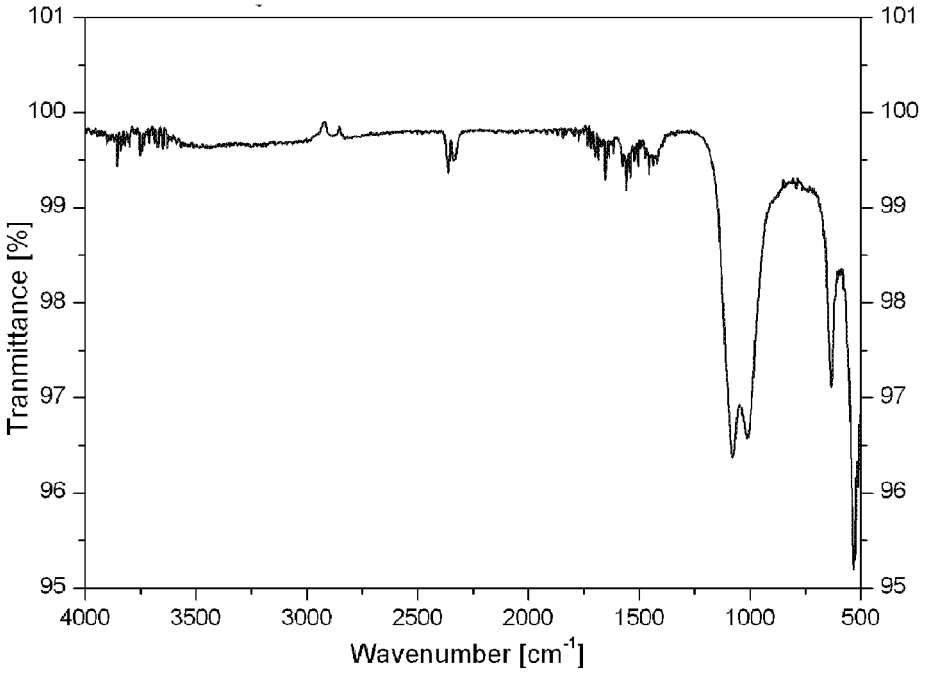
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Fig. 2

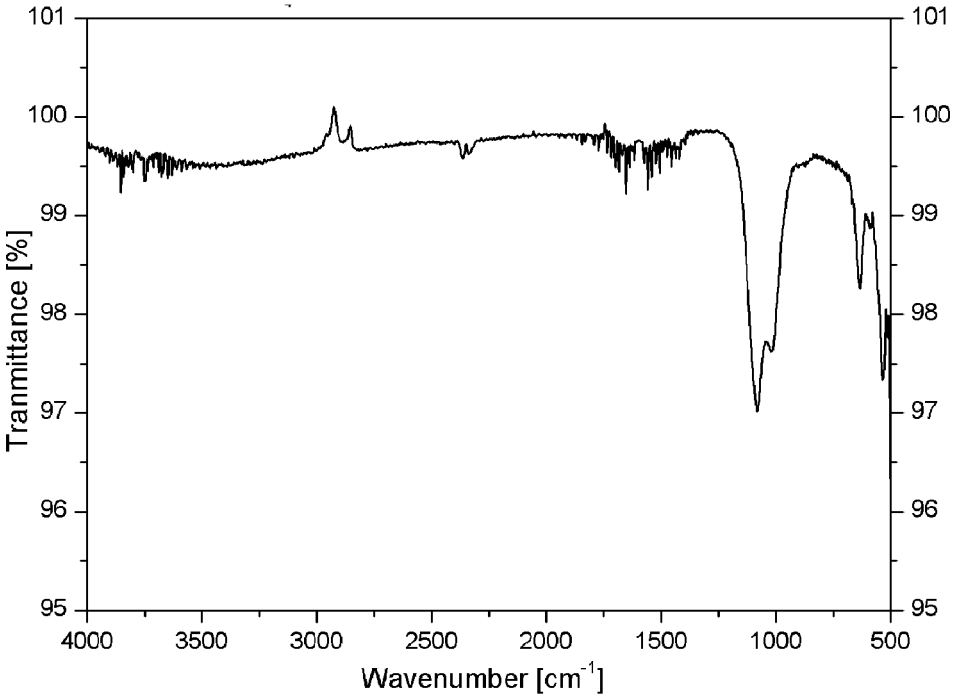
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Fig. 3

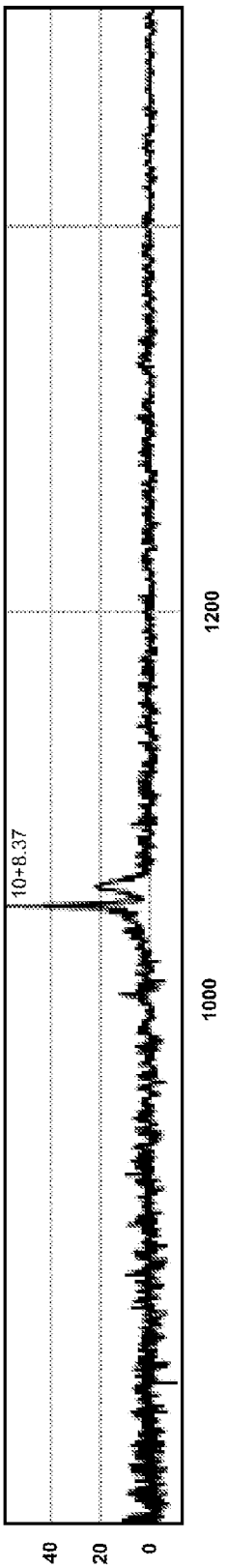

Fig. 4

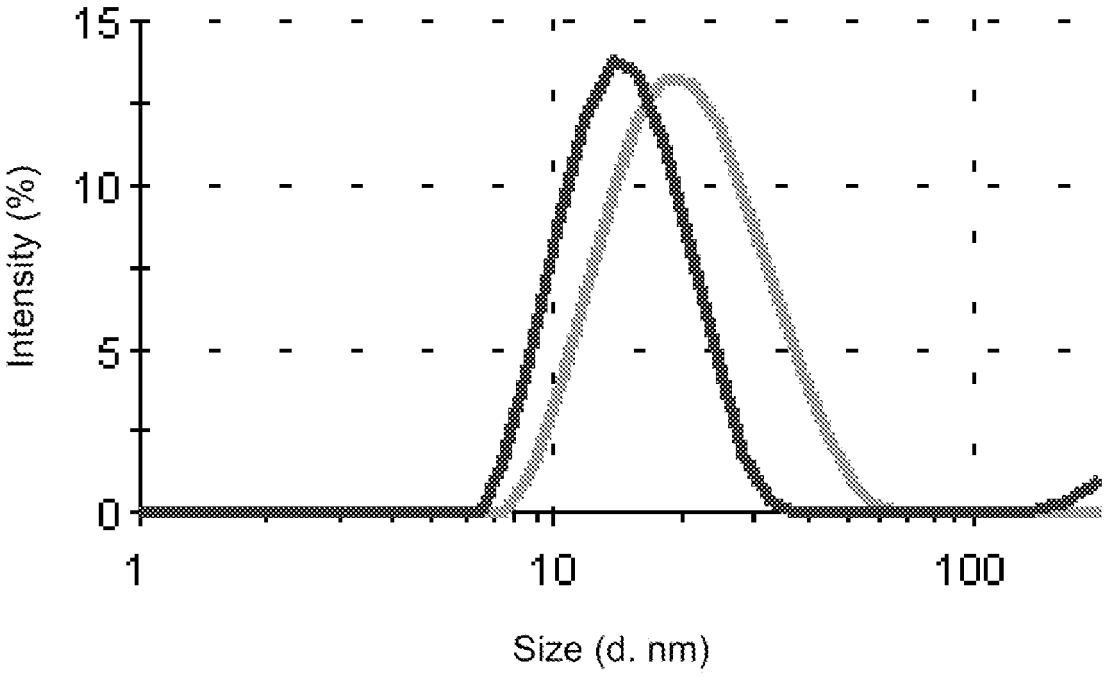

Fig. 5

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EUROPEAN SEARCH REPORT

Application Number
EP 10 17 2180

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	5 January 2011	Benfield, Alan

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05-01-2011

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