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

(11) EP 1 129 353 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
13.06.2007 Bulletin 2007/24

(21) Application number: **00959941.6**

(22) Date of filing: **06.09.2000**

(51) Int Cl.:
G01N 33/66 *(2006.01)*

(86) International application number:
PCT/US2000/024438

(87) International publication number:
WO 2001/018543 (15.03.2001 Gazette 2001/11)

(54) **MINIMALLY INVASIVE METHODS FOR MEASURING ANALYTES IN VIVO**

INVASIVES VERFAHREN ZUR IN VIVO ANALYTVERMESSUNG

PROCEDES DE MESURE D'ANALYTES IN VIVO AVEC EFFRACTION MINIMALE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **10.09.1999 US 393738**

(43) Date of publication of application:
05.09.2001 Bulletin 2001/36

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- **JAMES, T.D. ET AL.: "Novel saccharide-photoinduced electron transfer sensors based on the interaction of boronic acid and amine." J. AM.CHEM.SOC., vol. 117, 1995, pages 8982-8987, XP002156323 cited in the application**

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Description

BACKGROUND OF THE INVENTION

Area of the Art

[0001] The invention relates generally to methods of measuring an analyte contained in a body fluid and specifically to minimally invasive methods for measuring analytes, particularly glucose contained in an interstitial fluid of a body.

Description of the Prior Art

[0002] Treatment of diabetes requires frequent measurement of tissue glucose concentration. This is commonly accomplished by drawing a small blood sample (as by a fingerstick) several times daily. A patient typically uses a lancet to draw a droplet of blood and applies the droplet to a reagent strip which is read in a small meter. Obviously, the process is painful, invasive, time-consuming, and generally unpleasant.

[0003] WO-A-98/22820 describes fluorescent substances capable of undergoing a photo-induced electron transfer (PET) and generating a detectable analyte signal in response to the analyte (such as glucose) concentration in the fluid. The fluorescent substance is immobilized on a substrate placed into the skin in contact with the analyte. The substrate is preferably a biocompatible polymer. The fluorescent substance is either entrapped in a polymer matrix or covalently attached to and surrounded by the polymer matrix. The substrate may further comprise a calibration fluorophore, which provides a signal not interfering with the signal from the amplification components.

[0004] Extensive efforts have been made to measure blood glucose non-invasively However, proposed non-invasive methods, to date, rely on intensive signal massaging to extract a glucose signature from an overwhelming background. Therefore, it appears very difficult to provide a non-invasive measurement with the required specificity, accuracy, and precision.

[0005] The methods provided by the present invention provide a compromise between the conventional fingerstick techniques and the prospective non-invasive techniques. Methods of the present invention are able to preserve the diagnostic performance of more intrusive measurements without drawing samples but do require periodic replacement of passive implanted sensors.

SUMMARY OF THE INVENTION

[0006] It is an object of the present invention to develop an in vivo glucose measurement method that is as non-invasive as possible. It is also an object of the present invention to provide an *in vivo* glucose measurement that meets the clinically required specificity, accuracy, and precision.

[0007] Accordingly, the present invention as defined in the claims provides sensor particles for use in a method for detecting an analyte contained in the interstitial fluid of a body. The method comprises the steps of

(a) providing at least one sensor particle capable of generating a detectable analyte signal in responding to the analyte concentration in the body,

(b) placing the sensor particle into the skin of the body for allowing the sensor particle to be in contact with the interstitial fluid of the body to generate a detectable analyte signal,

(c) detecting the generated analyte signal, and

(d) determining the concentration of the analyte contained in the interstitial fluid.

[0008] The present invention may be used to measure the glucose concentration of the interstitial fluid in a human as a surrogate measurement for blood glucose. Preferably, the sensor particles are also capable of generating a detectable reference signal for background corrections.

[0009] In the present invention the sensor particles comprise a substance including a receptor and a signal fluor, wherein the receptor preferentially recognizes the analyte and is capable of binding to the analyte, and binding of the receptor to the analyte makes the substance undergo a photoinduced electron transfer (PET), whereby a detectable analyte signal is generated in response to the analyte concentration of the body, said signal fluor is responsive to the binding of the receptor to an analyte, such that signal fluor contained in analyte-bound receptor generates a first signal, and signal fluor contained in analyte-free receptor generates a second signal, a reference signal, wherein both signals are responsive to the concentration of the analyte contained in the interstitial fluid, whereby the first and second signals are distinguishable by their optical properties, wherein the receptor and signal fluor are on the same molecule; and further wherein the sensor particle also comprises another substance capable of generating a detectable reference signal independent of the analyte concentration in the interstitial fluid of a body.

[0010] Methods using sensor particles of the present invention are less intrusive than the conventional fingerstick technique for measuring blood glucose. They only require periodical reciprocal replacement of the sensor particles in the skin. In addition, since the sensor particles are in contact with the analytes, relatively specific chemical interactions may be used. The present invention therefore provides better performance than the proposed non-invasive methods for measuring blood glucose. The non-invasive methods rely on intensive signal massaging to extract a glucose signature from an overwhelming background.

[0011] The invention is defined in its fullest scope in the appended claims and is described in its preferred embodiments.

DESCRIPTION OF THE FIGURES

[0012] The above-mentioned and other features of this invention and the manner of obtaining them will become more apparent, and will be best understood, with reference to the following description, and accompanying drawings, in which:

FIGURE 1 is a cartoon of a receptor molecule with a signal fluor that are used in the present
FIGURE 2 shows the derailed suucture of a glucose receptor molecule
FIGURE 3 shows the interaction of the receptor of Figure 2 with glucose.
FIGURE 4 is a diagram depicting components that may be used to implement a method of providing a sensor particle.
FIGURE 5 is a plot of the fluorescence of a solution of the glucose receptor molecule against glucose concentration across the physiological range.

DETAILED DESCRIPTION OF THE INVENTION

[0013] The present invention as defined by the claims provides sensor particles for use in a method for detecting an analyte contained in the interstitial fluid of a body. The method comprises the steps of:

(a) providing at least one sensor particle capable of generating a detectable analyte signal in responding to the analyte concentration of the body,
(b) placing the sensor particle into the skin of the body for allowing the sensor particle to be in contact with the interstitial fluid of the body to generate the detectable analyte signal,
(c) detecting the generated analyte signal, and
(d) determining the concentration of the analyte contained in the interstitial fluid.

[0014] The analyte may be a glucose, and the concentration of the glucose in the interstitial fluid is determined as a measurement for the glucose concentration in the blood of the human. Glucose within the interstitial fluid is in equilibrium with blood glucose. AJthough there is a time lag of a few minutes before changes in blood glucose concentration are reflected in the interstitial fluid, this lag is negligible compared to the usual time between measurements. Thus, measurement of interstitial fluid glucose may be an adequate surrogate for measurement of capillary blood glucose.

[0015] For the purpose of the present invention, a body may be a body of a vertebrate animal. Examples of such an animal include but are not limited to: human; livestock such as cows, goats, sheep, chickens, etc.; and laboratory research animals such as rats, mice, rabbits, monkeys, etc. Preferably, the body is a human body.

[0016] In accordance with the present invention, the detectable analyte signal is an optically detectable signal. Preferably, the detectable signals are fluorescence signals. Most preferably, signal fluors contained in a sensor particle of the present invention emit in the near infrared (IR) and are relatively resistant to photo-bleaching. Near IR wavelengths are less readily absorbed or scattered by tissue.

[0017] The sensor particle may comprise a particle substrate bound to a receptor with a signal fluor. The receptor preferentially recognizes the analyte, and the binding of the receptor to the analyte allows the signal fluor to generate a detectable analyte signal that is responsive to the concentration of the analyte. The phrase "preferentially recognize" as used herein means that the receptor has a sufficiently higher affinity to the analyte than to other molecules of appreciable concentration contained in the interstitial fluid. The affinity is sufficiently higher if the signal due to binding of other molecules is negligible compared to the signal due to binding of the analyte. When the analyte is glucose, selectivity of a receptor is important but not crucial, since glucose is present at much higher concentration than potentially interfering saccharides.

[0018] It should be understood that the sufficiency of the affinity is determined by comparing the receptor affinity of an analyte to that of other molecules contained in a sample. Therefore, in some cases, a receptor that is capable of binding to different molecules with different affinities and that has relatively low affinity to an analyte may also be suitable for the purpose of the present invention, if the analyte is the only molecule that binds to the receptor and that has significant variation in its concentration in a sample. Therefore the bulk of the change in signal may be attributed to changes in analyte concentration, and the concentration of analyte may be determined once a baseline calibration is made.

[0019] Sensor particles that contain a collection of lower specificity receptors with distinguishable fluorescence may also be used. Each lower specificity receptor responds to binding of ane or more molecular species with different affinities. At least one lower specificity receptor must respond to binding of an analyte. If there are at least as many types of lower specificity receptors as there are molecular species with significant binding, the concentration of analyte may be calculated from the measured signals from each type of lower specificity receptor, and from *a priori* knowledge of the relative affinities of the lower specificity receptors for the different molecular species. This requires that the signals from each type of lower specificity receptor be distinguishable from one another. The signals may be distinguished by variations in optical properties of the signals. For example, if the signals are fluorescent, the optical property differences may be in emission wavelength, in excitation wavelength, in fluorescence lifetime, in polarization, in phase, or combina-

tions thereof.

[0020] It is preferred that the binding of the receptor to the analyte is reversible so that it may approach an equilibrium value and respond to changes in analyte concentration.

[0021] The sensor particle is also capable of generating one or more detectable reference signals that are distinguishable from the analyte signal. A reference signal may be distinguished from the analyte signal by optical properties. When the signals are fluorescent, the optical property differences may be in emission wavelength, in excitation wavelength, in fluorescence lifetime, in polarization, in phase, or in combinations thereof. Reference signals may be used to correct for variations in illuminating intensity and uniformity, illuminated area, overlying tissue optical density, fluor aging, and the like. Therefore, by comparing the analyte signal to the reference signal, one may correctly determine the concentration of the analyte contained in the interstitial fluid. It should be understood that reference signals may also encode information about the analyte concentration so that the concentration may be determined by combination of the analyte and reference signals or of their measurements.

[0022] The sensor particles may be made responsive to analyte concentration by incorporating specific analyte receptors with signal fluors, either onto the surface or into the body of the particles. For example, when an analyte is glucose, analyte receptors of the present invention may be dual diboronic acids conjugated to fluors, preferably where the fluors have excitation wavelength in the near infrared region (greater than 600nm). Examples of such dual diboronic acids include, but are not limited to, N,N-Bis(2-boronylbenryl)-3,3,3',3'-tetramethylindolinium chloride and 9,10-Bis[N-methyl-N-(o-boronobenzyl)amino]methyl]anthracene.

[0023] Examples of a receptor that preferentially recognizes a glucose analyte are also described by Tony James et al., in the Journal of the American Chemical Society, 1995, vol. 117 pp. 8982-8987, the relevant content of which is incorporated herein by reference. Briefly, these receptors use photo-induced electron transfer (PET) between an amine group and an incorporated anthracene fluor as modulated by binding of saccharide hydroxyls to a pair of boronic acids. The dual boronates confer a tenfold specificity to D-glucose as compared to D-fructose.

[0024] FIGURE 1 is a cartoon of a receptor molecule with a signal fluor adapted from the reference by Dr. Tony James. It shows: analyte binding groups, comprising spaced dual phenyl boronic acids conferring specificity for α-D-glucopyranose (glucose); a fluorescent group, coupled to the binding groups; and nitrogen atoms, with associated lone pair electrons coupled to the fluorescent group. In the absence of glucose binding (upper portion of figure), the fluorescence by the fluorescent group is quenched. When glucose is bound (lower portion), fluorescence is enhanced. The detailed structure of the glucose receptor molecule, as described in the reference by Tony James, is shown in Figure 2. Figure 3 shows the interaction of the receptor of Figure 2 with glucose as described in the reference by Tony James.

[0025] The analyte signal and the reference signal may be generated by the same molecule or by different molecules contained in or on the sensor particles. For example, the sensor particle may contain a receptor with both a signal fluor and a reference fluor. The receptor preferentially recognizes the analyte. When the receptor is bound to the analyte, the signal fluor will generate a detectable signal that is responsive to the concentration of the analyte contained in the interstitial fluid. However, the signal generated by the reference fluor will not be affected by the binding of the receptor to the analyte.

[0026] Alternatively, a receptor with one signal fluor may be used. The signal fluor is responsive to the binding of the receptor to an analyte. In this case, the receptor that is bound to the analyte is a bound receptor and the receptor that is not bound to the analyte is a free receptor. The signal fluor generates a first signal when it is contained in a bound receptor. It generates a second signal when it is contained in a free receptor. The first and second signals are distinguishable in their optical properties. In this case, the reference signal, the second signal, also encodes information about the analyte concentration. Both signals are responsive to the concentration of the analyte contained in the interstitial fluid, as the relative distribution of bound and free receptors is controlled by a chemical equilibrium governed by the analyte concentration. Measurements of these two signals may be mathematically combined to determine analyte concentration that, except for random fluctuations, is independent of the excitation light level, the optical transmission path, and the absolute number or activity of the sensor particles. A necessary condition for this to occur is for the sensor particles to not significantly alter the concentration of analyte in the interstitial fluid. This may be accomplished by keeping the number of bound receptors small compared to the number of analyte molecules. This condition is easily achieved for analytes of reasonable concentration.

[0027] Further, the sensor particle may include a first substance capable of generating a signal that is responsive to the analyte concentration of a body and a second substance capable of generating a reference signal that is independent of the analyte concentration of a body. In other words, the reference fluor may be a separate molecule as to the signal fluor that is responsive to the analyte concentration. For example, the first substance may be a receptor which preferentially recognizes the analyte, and the second substance may be a fluorescent molecule that is not capable of binding to a receptor. Examples of such a fluorescent molecule include, but are not limited to, oxazine 750, IR140, IR143 and IR144, all of which are commercially available from Exciton Inc. of Dayton Ohio; Cy2, Cy3, Cy3.5, Cy5, Cy5.5 and Cy7, all of which are commercially available from Amersham Pharmacia

Biotech of Sweden and the United Kingdom. Additional examples of fluorescent molecules include phthallocyanine dyes, LaJolla Blue (Si phthallocyanine with PEG axial ligands), fluorescein isothiocyanate ("FITC"), rhodamine isothiocyanate; 2, 4-dinitrofluorobenzene, phenylisothiocyanate, dansyl choloride, substituted rhodamine isothiocyanates ("XRITC"), tetraethyl rhodamine isothiocyanate ("TRITC"), and phycobiliproteins (e.g., allophycocyanin, HDITCP (1,1', 3.3.3', 3' hexamethyl- 4,4', 5.5' dibenzo-2.2' indotricar bocyanine percholoate), and phycoerythrin) fluorophores, discussed in U.S. Patent No. 4,877,965.

[0028] The reference fluor may be the same molecule as the signal fluor; the reference thereby gives a measure of the receptor integrity. In such a case, it is required that signals from the bound and the free receptors are optically distinguishable. For example, when the signals are fluorescent, the optical property differences may be in emission wavelength, in excitation wavelength, in fluorescence lifetime, in polarization, in phase, or in combinations thereof However, when the reference fluor is a separate molecule, it can still correct for variations in illumination intensity and light path but can only indirectly correct for sensor particle aging due to photo-bleaching.

[0029] The sensor particle of the present invention may be a hydrophilic particle such as, but not limited to, controlled pore glass (CPG) beads or a polymer gel. It may also be a hydrophobic particle with appropriate plasticizers to permit free permeation by small analytes. Alternatively, it may be a semipermeable membrane such as, but not limited to, a liposome. To avoid the degradation of the receptor, the receptor may be bound to the inside of a hydrophilic particle, such as pores of CPG beads or a polymer gel. The receptors may also be captured inside a hydrophobic particle with appropriate plasticizers to permit free permeation by small analytes. The receptor may further be packaged inside the semipermeable membrane. For the purpose of the present invention, a plasticizer is appropriate if it permits free permeation of small analytes into a hydrophobic particle. Examples of such a plasticizer include, but are not limited to, Dioctyl Adipate, Diisodecyl Adipate, and the like. A receptor of the present invention may also be bound to the surface of hydrophobic or other insoluble particles.

[0030] The particle sensors of the present invention are in a size that prevents their encapsulation by scar tissues. Preferably, the particle sensors are built on the same scale as cells in the body. In an embodiment of the present invention, the sensor panicles are in a size range from about 1-10 micrometers.

[0031] The particles are preferably round and uniform, such as commonly available polystyrene latex particles formed by emulsion polymerization. They may be produced of other materials and by other processes that are known in the art Examples of the materials and methods include, but are not limited to, plasticized polyvinyl chloride (PVC) particles produced by droplet casting of dissolved polymers or glass-like particles produced from sol gels. In addition, the sensor particles may be made of a bio-resorbable polymer. Examples of a bio-resorbable polymer include, but are not limited to, polyglycoic acid (PGA), poly-DL-lactide-co-glycolide (PLGA), starch, gelatin, and the like.

[0032] The sensor particles of the present invention may be placed into the skin by any method that allows the sensor particles to be in contact with the interstitial fluid. For example, the sensor particles may be tattooed to the skin. If the sensor particles are made of bio-absorbable materials, they may be placed in contact with the skin under a condition that allows the skin to absorb the bio-absorbable polymer into the skin.

[0033] Once the sensor particles are placed in contact with the interstitial fluid, a laser light source may be used to emit laser light for exciting signal fluors. A laser light source can be a near-infrared (NIR) laser, a visible light laser or ultraviolet laser, although the NIR laser is preferred. Laser diodes may be used as a light source since they are inexpensive, compact, high-intensity sources of exciting light for signal fluors of the present invention. Commercially available laser diodes that are suitable for use in the present invention include, but are not limited to, the diode laser sold by Toshiba America Electronics Components, Inc. as Model Numbers TOLD9211F and TOLD9441MC.

[0034] The invention uses an external meter to detect analyte and reference signals. This meter may be held continuously in proximity to the skin overlying the sensor particles or, more preferably, it may be brought into such proximity at such time as analyte determinations are desired. The analyte and reference signals may be detected by methods commonly known in the art, once a sensor particle of the present invention is placed into the skin and the sensor particle is in contact with the interstitial fluid. In accordance with one embodiment of the present invention, an external meter shines light onto the skin and collects returned light from the skin. This returned light includes components backscattered by the tissue and by the particles, plus a portion of the signal and reference light produced by the particles. The meter measures the returned portion of signal and reference light by methods commonly known in the art, such as the use of wavelength selective filters, phase sensitive detection, or time gated detection. The meter may take multiple measurements of signal and reference light during a single determination, thereby reducing the effects of random fluctuations and other noise sources. The meter may combine the measurements of signal fluorescent light with the measurements of reference fluorescence light to correct for variations in illuminating intensity, overlying tissue optical density, fluor aging, and the like. One example of such a meter includes: a source, preferably a laser diode; a pair of detectors such as photomultiplier tubes, avalanche photodetectors, or photodiodes; a pair of optical filters, each associated with one of the detectors, to separate the emitted light by wavelength; a collection optics to direct received light through the filters

and onto the photodetectors; and a controller to determine levels based on the strength of the measured signals.

[0035] In order that the meter may be properly aligned with that region of the skin which overlies the sensor particles, it is desirable that the region be marked so that the user may readily locate it. Such marking can be applied with a surface marker, such as a pen, or the marking may be applied as part of the sensor implant. The receptor molecules themselves may act as a marker, but it is expected that this will not prove adequate given the limited quantity of receptor molecules implanted and the preferred near IR wavelengths. The particles may be attached to or loaded with a more readily visible dye or pigment, or they may be co-implanted with other particles attached to or loaded with such a dye or pigment.

[0036] Periodically, the sensor particles are replaced with new particles. The sensor particles must be periodically replaced as they age due to photo-bleaching, to resorption by the body, or to other degradation processes. Replacement of sensor particles may be carried out by a health care professional.

[0037] Figure 4 is a diagram depicting components that may be used to implement a method of providing a sensor particle as described herein. In Figure 4, sensor particles 1 are implanted into the skin 2. After the implantation, excitation light 3 is transmitted through the intervening skin portions from outside of the body to excite the signal fluors contained in the sensor particles, As a result, signal light 4 is emitted by the sensor particles in response to the excitation light. A portion of the signal light 4 is detected by detection optics 5 for measuring a portion of the signal light transmitted through the intervening portions of the skin. An analysis unit 6 is used for converting the signal light measurements into determinations of glucose concentration.

[0038] FIGURE 5 is a plot of the fluorescence of a solution of the glucose receptor molecule of Figure 2 against glucose concentration across the physiological range. The glucose receptor molecule is dissolved in diluted, glucose-depleted caprine serum as a simulant for the interstitial milieu Figure 5 demonstrated that the glucose concentration of the interstitial milieu may be determined by measuring the fluorescence intensity of the signal fluors associated with glucose receptor molecules.

[0039] The foregoing is meant to illustrate, but not to limit, the scope of the invention. Indeed, those of ordinary skill in the art can readily envision and produce further embodiments, based on the teachings herein, without undue experimentation.

[0040] The present invention may be embodied in other specific forms without departing from its essential characteristics. The described embodiment is to be considered in all respects only as illustrative and not as restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of the equivalence of the claims are to be embraced within their scope.

Claims

1. A sensor particle for use in a method for detecting an analyte contained in the interstitial fluid of a body, comprising a substance including a receptor and a signal fluor, wherein the receptor preferentially recognizes the analyte and is capable of binding to the analyte, and binding of the receptor to the analyte makes the substance undergo a photoinduced electron transfer (PET), whereby a detectable analyte signal is generated in response to the analyte concentration of the body, said signal fluor is responsive to the binding of the receptor to an analyte, such that signal fluor contained in analyte-bound receptor generates a first signal, and signal fluor contained in analyte-free receptor generates a second signal, a reference signal, wherein both signals are responsive to the concentration of the analyte contained in the interstitial fluid, whereby the first and second signals are distinguishable by their optical properties, wherein the receptor and signal fluor are on the same molecule; and further wherein the sensor particle also comprises another substance capable of generating a detectable reference signal independent of the analyte concentration in the interstitial fluid of a body.

2. The particle of claim 1, wherein the sensor particle is selected from a hydrophilic particle with the receptor bound to the inside of the particle, a hydrophobic particle with the receptor captured inside the particle, and a hydrophobic insoluble particle with the receptor coupled to the surface of the particle.

3. The particle of claim 1, wherein the sensor particle comprises at least two different types of receptors with signal fluors, and the receptors preferentially recognize the analyte.

4. The particle of claim 1 wherein the first substance includes different receptors with signal fluors, and the receptors preferentially recognize the analyte.

5. The particle of claim 1, wherein the sensor particle is made of a bio-resorbable polymer, and the bio-resorbable polymer is selected from polyglycolic acid (PGA), poly-DL-lactide-co-glycolide (PLGA), starch, gelatin, and the like.

6. The particle of claim 1, wherein the analyte is glucose, and the receptors preferentially recognize glucose, and at least one receptor is diboronic acid conjugated to the signal fluor.

7. The particle of claim 6, wherein the diboronic acid is

9,10-bis[N-methyl-N-(*o*-boronobenzyl)amino] methyl]anthracene.

8. The particle of claim 2, in which the sensor particle comprises a hydrophilic particle and the receptor is bound to the inside of the hydrophilic particle wherein the hydrophilic particle is a CPG glass bead or a polymer gel, and the receptor is bound to the inside of the pores of the CPG glass bead or the pores of the polymer gel.

9. The particle of claim 2, wherein the sensor particle comprises a hydrophobic material including appropriate plasticizers to permit free permeation by the analyte contained in the interstitial fluid of a body, and wherein the receptor is captured inside the hydrophobic particle.

10. The particle of claim 1, wherein the sensor particle comprises a semi-permeable membrane, and the receptor is packaged inside the semi-permeable membrane.

11. The particle of claim 10, wherein the semi-permeable membrane is a liposome.

12. The particle according to any one of the preceding claims, wherein the size of the sensor particle is in the range of from 1 to 10 microns.

13. The particle of claim 12, wherein the sensor particle is of a uniform shape.

14. The particle of claim 13 wherein the sensor particle is of a round shape.

15. The particle of claim 1, wherein the sensor particle is made from a material selected from polystyrene latex particles, plasticized polyvinyl chloride particles, glass-like particles, a semi-permeable membrane, and a bio-resorbable polymer.

Patentansprüche

1. Sensorteilchen zur Verwendung in einem Verfahren zum Bestimmen eines Analyten, der in der interstitiellen Flüssigkeit eines Körpers enthalten ist, umfassend eine Substanz, die einen Rezeptor und ein Signalfluophor einschließt, wobei der Rezeptor vorzugsweise den Analyten erkennt und befähigt ist, an den Analyten zu binden, und wobei das Binden des Rezeptors an den Analyten dazu führt, daß die Substanz einen photoinduzierten Elektronentransfer (PET) eingeht, wodurch ein detektierbares Analytensignal in Antwort auf die Analytenkonzentration des Körpers erzeugt wird, wobei der Signalfluophor auf das Binden des Rezeptors an einen Analyten anspricht, so daß der in dem Analyt-gebundenen Rezeptor enthaltene Signalfluophor ein erstes Signal erzeugt, und der in dem Analyt-freien Rezeptor enthaltene Signalfluophor ein zweites Signal, ein Referenzsignal, erzeugt, wobei beide Signale auf die Konzentration des in der interstitiellen Flüssigkeit enthaltenen Analyten ansprechen, wobei das erste und zweite Signal durch deren optische Eigenschaften unterscheidbar sind, wobei der Rezeptor und der Signalfluophor an dem gleichen Molekül sind, und wobei weiter das Sensorteilchen auch eine weitere Substanz umfaßt, die befähigt ist, ein detektierbares Referenzsignal, unabhängig von der Analytenkonzentration in der interstitiellen Flüssigkeit eines Körpers, zu erzeugen.

2. Teilchen gemäß Anspruch 1, wobei das Sensorteilchen aus einem hydrophilen Teilchen mit dem Rezeptor gebunden an das Innere des Teilchens, einem hydrophoben Teilchen mit dem Rezeptor gefangen innerhalb des Teilchens und einem hydrophoben unlöslichen Teilchen mit dem Rezeptor gekoppelt an die Oberfläche des Teilchens ausgewählt ist.

3. Teilchen gemäß Anspruch 1, wobei das Sensorteilchen mindestens zwei unterschiedliche Typen von Rezeptoren mit Signalfluophoren umfaßt, und die Rezeptoren vorzugsweise den Analyten erkennen.

4. Teilchen gemäß Anspruch 1, wobei die erste Substanz verschiedene Rezeptoren mit Signalfluophoren einschließt, und die Rezeptoren vorzugsweise den Analyten erkennen.

5. Teilchen gemäß Anspruch 1, wobei das Sensorteilchen aus einem bioresorbierbaren Polymer ist, und das bioresorbierbare Polymer aus Polyglykolsäure (PGA), Poly-DL-lactid-co-glycolid (PLGA), Stärke, Gelatin und dergleichen ausgewählt ist.

6. Teilchen gemäß Anspruch 1, wobei der Analyt Glucose ist, und die Rezeptoren vorzugsweise Glucose erkennen, und mindestens ein Rezeptor Diboronsäure, konjugiert an den Signalfluophor, ist.

7. Teilchen gemäß Anspruch 6, wobei die Diboronsäure 9,10-Bis[N-methyl-N-(*o*-boronbenzyl)amino]methyl]anthracen ist.

8. Teilchen gemäß Anspruch 2, worin das Sensorteilchen ein hydrophiles Teilchen umfaßt, und der Rezeptor an das Innere des hydrophilen Teilchens gebunden ist, wobei das hydrophile Teilchen ein CPG-Glaskügelchen oder ein Polymergel ist, und der Rezeptor an das Innere der Poren der CPG-Glaskügelchen oder die Poren des Polymergels gebunden ist.

9. Teilchen gemäß Anspruch 2, wobei das Sensorteilchen ein hydrophobes Material umfaßt, welches geeignete Weichmacher einschließt, um freie Permeation durch den in der interstitiellen Flüssigkeit eines Körpers enthaltenen Analyten zu erlauben, und wobei der Rezeptor innen in dem hydrophoben Teilchen gefangen ist.

10. Teilchen gemäß Anspruch 1, wobei das Sensorteilchen eine semi-durchlässige Membran umfaßt, und der Rezeptor innen in der semi-durchlässigen Membran eingepackt ist.

11. Teilchen gemäß Anspruch 10, wobei die semi-durchlässige Membran ein Liposom ist.

12. Teilchen gemäß einem der vorhergehenden Ansprüche, wobei die Größe des Sensorteilchens in dem Bereich von 1 bis 10 μm liegt.

13. Teilchen gemäß Anspruch 12, wobei das Sensorteilchen von einer einheitlichen Form ist.

14. Teilchen gemäß Anspruch 13, wobei das Sensorteilchen von einer runden Form ist.

15. Teilchen gemäß Anspruch 1, wobei das Sensorteilchen aus einem Material, ausgewählt aus Polystyrollatexteilchen, weichgemachten Polyvinylchloridteilchen, Glas-ähnlichen Teilchen, einer semi-durchlässigen Membran und einem bio-resorbierbaren Polymer, hergestellt ist.

Revendications

1. Particule sonde destinée à être utilisée dans une méthode permettant de détecter un analyte contenu dans le fluide interstitiel d'un corps, comprenant une substance incluant un récepteur et un composé signal fluorescent, dans laquelle le récepteur reconnaît de préférence l'analyte et est capable de se lier à l'analyte, et la liaison du récepteur avec l'analyte fait subir à la substance un transfert d'électrons photoinduit (TEP), moyennant quoi un signal d'analyte détectable est généré en réponse à la concentration en analyte du corps, ledit composé signal fluorescent réagit à la liaison du récepteur avec un analyte, de telle sorte que le composé signal fluorescent contenu dans le récepteur lié à l'analyte génère un premier signal, et le composé signal fluorescent contenu dans le récepteur exempt d'analyte génère un deuxième signal, un signal de référence, dans laquelle les deux signaux réagissent à la concentration de l'analyte contenu dans le fluide interstitiel, moyennant quoi les premier et deuxième signaux peuvent se distinguer grâce à leurs propriétés optiques, dans laquelle le récepteur et le composé signal fluorescent se trouvent sur la même molécule ; et en outre dans laquelle la particule sonde comprend également une autre substance capable de générer un signal de référence détectable indépendant de la concentration en analyte du fluide interstitiel d'un corps.

2. Particule selon la revendication 1, dans laquelle la particule sonde est choisie entre une particule hydrophile, le récepteur étant lié à l'intérieur de la particule, une particule hydrophobe, le récepteur étant capturé à l'intérieur de la particule, et une particule insoluble hydrophobe, le récepteur étant couplé à la surface de la particule.

3. Particule selon la revendication 1, dans laquelle la particule sonde comprend au moins deux types différents de récepteurs avec des composés signaux fluorescents, et les récepteurs reconnaissent de préférence l'analyte.

4. Particule selon la revendication 1, dans laquelle la première substance inclut différents récepteurs avec des composés signaux fluorescents, et les récepteurs reconnaissent de préférence l'analyte.

5. Particule selon 1a revendication 1, dans laquelle la particule sonde est constituée d'un polymère biorésorbable, et le polymère biorésorbable est choisi entre l'acide polyglycolique (PGA), le poly-DL-lactide-co-glycolide (PLGA), l'amidon, la gélatine et similaire.

6. Particule selon la revendication 1, dans laquelle l'analyte est du glucose, et les récepteurs reconnaissent de préférence le glucose, et au moins un récepteur est de l'acide diboronique conjugué au composé signal fluorescent.

7. Particule selon la revendication 6, dans laquelle l'acide diboronique est le 9,10-bis[N-méthyl-N-(*o*-boronobenzyl)amino]méthyl]anthracène.

8. Particule selon la revendication 2, dans laquelle la particule sonde comprend une particule hydrophile et le récepteur est lié à l'intérieur de la particule hydrophile, dans laquelle la particule hydrophile est une bille de verre CPG ou un gel polymère, et le récepteur est lié à l'intérieur des pores de la bille de verre CPG ou des pores du gel polymère.

9. Particule selon la revendication 2, dans laquelle la particule sonde comprend une matière hydrophobe incluant des plastifiants appropriés afin de permettre une perméation libre par l'analyte contenu dans le fluide interstitiel d'un corps, et dans laquelle le récepteur est capturé à l'intérieur de la particule hydrophobe.

10. Particule selon la revendication 1, dans laquelle la particule sonde comprend une membrane semi-perméable, et le récepteur est intégré à l'intérieur de la membrane semi-perméable.

11. Particule selon la revendication 10, dans laquelle la membrane semi-perméable est un liposome.

12. Particule selon l'une quelconque des revendications précédentes, dans laquelle la taille de la particule sonde se trouve dans la plage comprise entre 1 et 10 microns.

13. Particule selon la revendication 12, dans laquelle la particule sonde présente une forme uniforme.

14. Particule selon la revendication 13, dans laquelle la particule sonde a une forme ronde.

15. Particule selon la revendication 1, dans laquelle la particule sonde est constituée d'une matière choisie entre les particules de latex polystyrène, particules de polychlorure de vinyle plastifié, particules de type verre, membrane semi-perméable, et polymère biorésorbable.

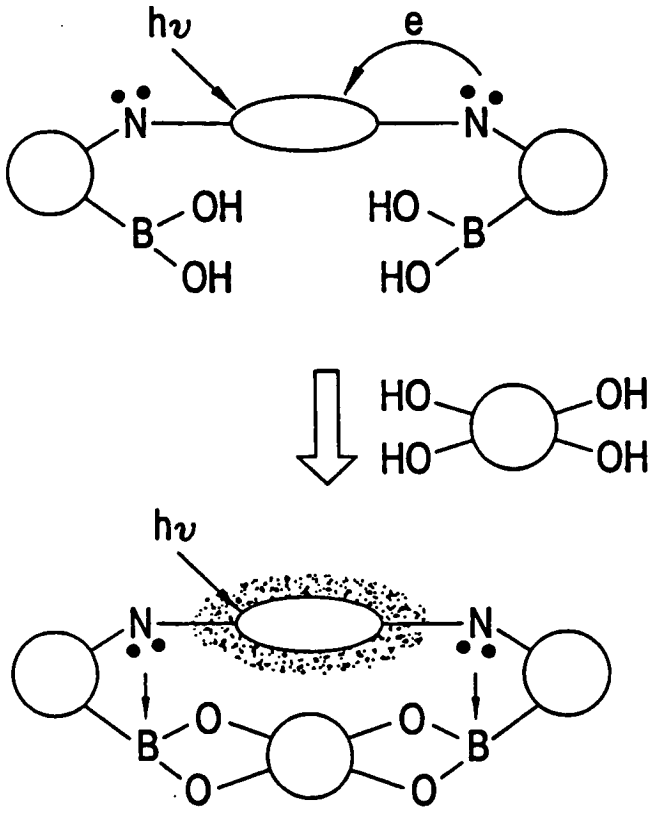

Fig. 1

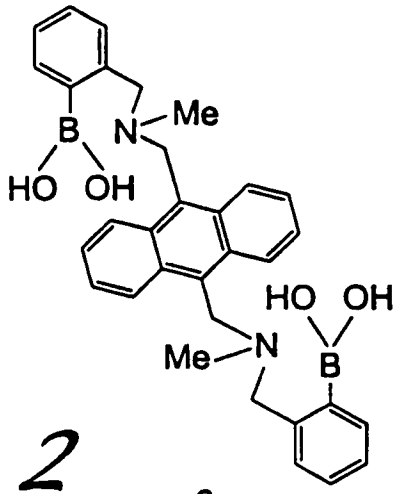

Fig. 2

8

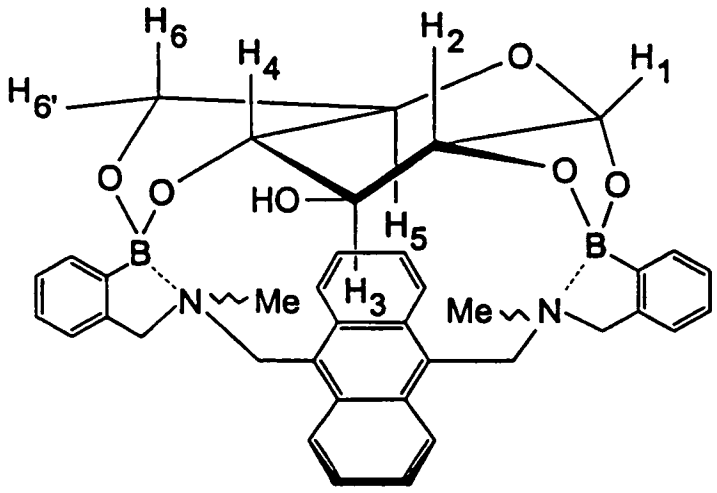

Fig. 3

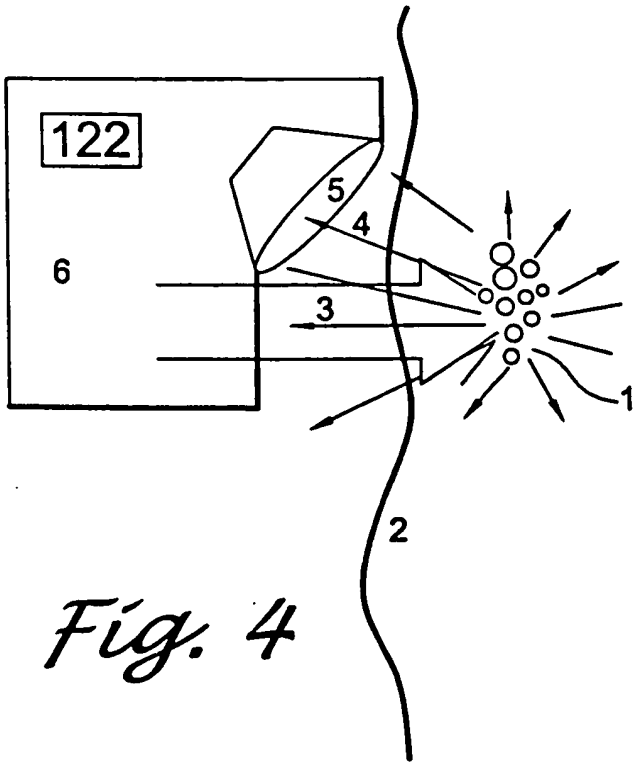
122
6
5
4
3
1
2

Fig. 4

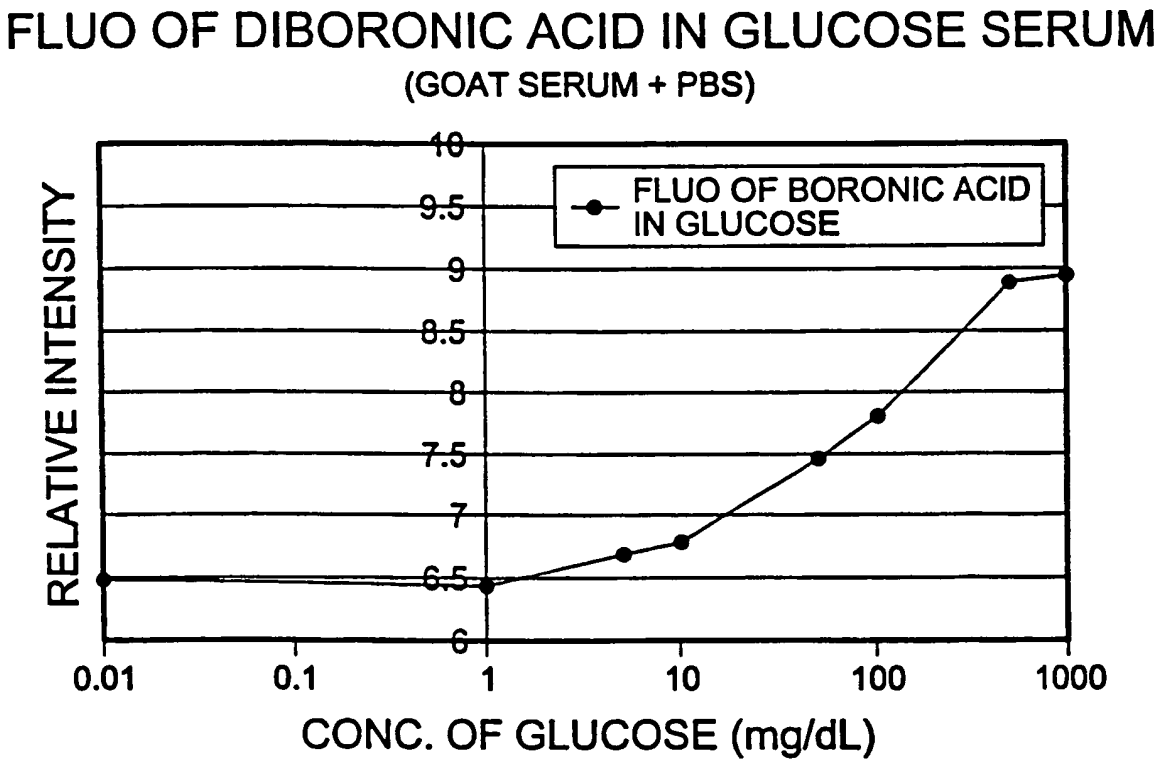
FLUO OF DIBORONIC ACID IN GLUCOSE SERUM
(GOAT SERUM + PBS)
RELATIVE INTENSITY
10
9.5
9
8.5
8
7.5
7
6.5
6
FLUO OF BORONIC ACID IN GLUCOSE
0.01
0.1
1
10
100
1000
CONC. OF GLUCOSE (mg/dL)

Fig. 5

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 9822820 A **[0003]**
- US 4877965 A **[0027]**

Non-patent literature cited in the description

- **TONY JAMES et al.** *Journal of the American Chemical Society,* 1995, vol. 117, 8982-8987 **[0023]**

专利名称(译)	用于测量体内分析物的微创方法		
公开(公告)号	EP1129353B1	公开(公告)日	2007-06-13
申请号	EP2000959941	申请日	2000-09-06
[标]申请(专利权)人(译)	贝克曼考尔特公司		
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IPC分类号	G01N33/66 G01N21/64 A61B5/00 A61B5/145 A61B5/1459 G01N21/78		
CPC分类号	G01N33/66 Y10S436/80		
优先权	09/393738 1999-09-10 US		
其他公开文献	EP1129353A1		
外部链接	Espacenet		

摘要(译)

提供了用于测量包含在身体的组织液中的分析物（例如葡萄糖）的微创方法。该方法包括以下步骤：（a）提供至少一个能够响应于身体的分析物浓度产生可检测的分析物信号的传感器颗粒，（b）将传感器颗粒放入身体的皮肤中以允许传感器颗粒与身体的组织间液接触以产生可检测的分析物信号，（c）检测产生的分析物信号，和（d）确定组织液中包含的分析物的浓度。通过包括对传感器颗粒中的分析物特异的光诱导电子转移受体，可以使传感器颗粒响应于分析物，例如体液中包含的葡萄糖浓度。

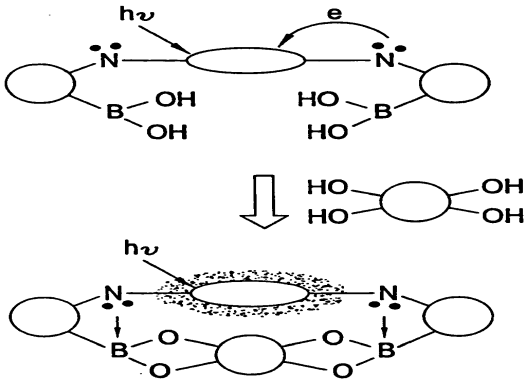

Fig. 1