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(54) Title: INDIVIDUALLY AND FLEXIBLY DEPLOYABLE TARGET-ANALYTE SENSITIVE PARTICULATE PROBES AND METHOD OF MAKING AND USING

(57) Abstract: Flexibly deployable, discrete, target-analyte sensitive particulate probes and methods of manufacturing and using. The probes each comprise a porous scaffold particle coated with an optically-active, target-analyte sensitive material. The scaffold particle has at least one of (i) a volume of 0.5 to 500 mm^3, and (ii) a largest dimension of 2 to 20 mm.

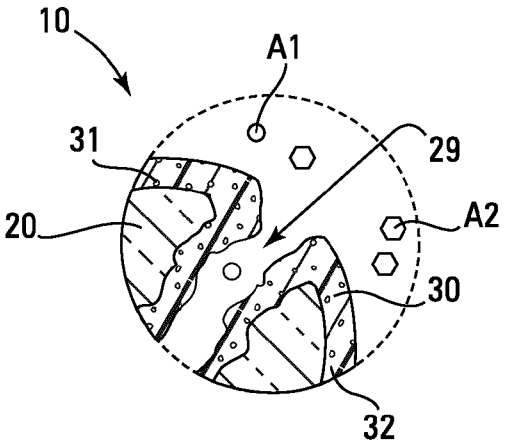

Fig. 2

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INDIVIDUALLY AND FLEXIBLY DEPLOYABLE TARGET-ANALYTE SENSITIVE PARTICULATE PROBES AND METHODS OF MAKING AND USING

BACKGROUND

[0001] Optically-active, target-analyte sensitive indicator dyes and compounded materials containing such dyes are widely used in the construction of probes and sensors for quantification and monitoring of target-analytes. Such sensors are particularly suited for use in those situations where nondestructive and/or continuous quantification and/or monitoring of a target-analyte within an enclosed space is necessary or desired as such sensors are amenable to repetitive, non-invasive and contactless interrogation through a variety of common barrier materials.

[0002] Sensors employing an optically-active, target-analyte sensitive indicator dye commonly immobilize the dye by embedding the dye within a polymer matrix that is permeable to the target-analyte, hereinafter referenced as an optically-active indicator matrix.

[0003] To facilitate handling and use, and avoid contamination of the sample being tested, the optically-active indicator matrix is commonly deposited as a solid-state coating, film, layer or dot on an appropriate substrate support material to form autonomously deployable sensors. See for example United States Published Patent Applications 2011/0136247, 2009/0029402, 2008/199360, 2008/190172, 2007/0042412, and 2004/0033575; United States Patents 8,242,162, 8,158,438, 7,862,770, 7,849,729, 7,749,768, 7,679,745, 7,674,626, 7,569,395, 7,534,615, 7,368,153, 7,138,270, 6,989,246, 6,689,438, 6,395,506, 6,379,969, 6,080,574, 5,885,843, 5,863,460, 5,718,842, 5,595,708, 5,567,598, 5,462,879, 5,407,892, 5,114,676, 5,094,959, 5,030,420, 4,965,087, 4,810,655, and 4,476,870; PCT International Published Application WO 2008/146087; and European Published Patent Application EP 1134583. Such optical sensors are available from a number of suppliers, including Presens Precision Sensing, GmbH of Regensburg, Germany, Oxysense of Dallas, Texas, USA, and Luxcel Biosciences, Ltd of Cork, Ireland.

[0004] Due to a prolific increase in the use of such optical sensors, manufacturers have begun to supply assay vessels and packaging films with an integrated optically-active indicator sensor, thereby facilitating use of such sensors by the end user, particularly those who assay large numbers of test samples on a regular basis. Examples of commercially available assay vessels with an integrated optically-active sensor include microtitter plates

available from BD Biosciences of Franklin Lakes, New Jersey, USA and PreSens – Precision Sensing GmbH of Regensberg, Germany; disposable plastic vials available from Mocon of Minneapolis, Minnesota, USA and Luxcel Biosciences, Ltd. of Cork, Ireland, and culturing flasks available from PreSense.

[0005] Such optically active sensors are usually integrated into assay vessels by depositing a solution or suspension of the optically-active indicator matrix directly onto an inner surface of the assay vessel as a polymeric 'cocktail', or adhesively attaching a solid state sensor to an inner surface of the assay vessel.

[0006] While constituting a significant advance and finding widespread acceptance within the industry, the manufacture of such sensor-integrated assay vessels is difficult as it requires precision deposition of small aliquots (typically nl and µl quantities) of a viscous cocktail on the inner surface of widely variable and often diminutive assay vessels. Inaccurate or inconsistent size, shape or location of the optically-active indicator matrix, as well as the drying/curing rate of the deposited matrix can result in significant variability in working properties of the resulting sensors. Hence, such sensor-integrated assay vessels are generally expensive to manufacture. Furthermore, use of sensor-integrated assay vessels restricts the end user to use of only those types of assay vessels available with an integrated sensor, thereby reducing the ability to change or adjust experimental conditions in terms of the assay vessel type, size, geometry, alignment, material of construction, etc. Due to high start-up production costs, and strong but limited demand for all but a few types of "sensorized" assay vessels, the industry is unlikely to significantly expand the types of assay vessels available with an integrated sensor.

[0007] One alternative for avoiding the drawbacks associated with the deployment of solid-state optically active sensors, is to employ fluid compositions containing the indicator dye in solution or suspension as a liquid or in the form of solid state nano or micro particles which are added to and blended into the samples being tested. This allows the sensor material to be supplied separately from the assay vessels, thereby facilitating a more versatile use relative to the integrated solid state sensors. However, such sensors contaminate the sample and require the use of a much greater quantity of indicator dye as the dye is diluted by the sample.

[0008] Accordingly, a substantial need exists for an optically-active, target-analyte sensitive probe capable of quick, easy, flexible and cost effective deployment in a wide

variety of assay vessels while consistently and reliably providing sensitive,d accurate and convenient target-analyte measurements.

SUMMARY OF THE INVENTION

[0009] A first aspect of the invention is a flexibly deployable, discrete target-analyte sensitive probe . The probe comprises a porous scaffold particle coated with an optically-active, target-analyte sensitive material. The scaffold particle has at least one of (i) a volume of 0.5 to 500 mm^3, and (ii) a largest dimension of 2 to 20 mm. In a preferred embodiment the probe has a density of greater than 1.2 g/cm^3 so that the probe, when introduced into an assay vessel will remain at or sink to the bottom of the vessel when combined with common test samples.

[0010] A second aspect of the invention is an article of commerce comprising a plurality of probes in accordance with the first aspect of the invention retained as a commingled supply of probes within a container from which the probes may be individually and discretely dispensed and used. In a preferred embodiment the probes are microbially sanitized probes.

[0011] A third aspect of the invention is a method for measuring concentration of a target-analyte within an enclosed space employing a probe according to the first aspect of the invention. The method includes the steps of (A) obtaining a supply of the target-analyte sensitive probes according to the first aspect of the invention, (B) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe, (C) enclosing the space, and (D) ascertaining target-analyte concentration within the enclosed space by: (i) exposing the sensor to excitation radiation to create an excited sensor, (ii) measuring radiation emitted by the excited sensor, and (iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

[0012] A fourth aspect of the invention is a method for measuring concentration of a target-analyte within the chamber of a plurality of receptacles employing probes dispensed from the second aspect of the invention. The method includes the steps of (A) obtaining an article of commerce according to the second aspect of the invention, (B) dispensing a first

known number of probes from the container, constituting a first fraction of the total number of commingled probes, into a chamber defined by a first receptacle to form a first sensor comprised of at least one probe, (C) dispensing a second known number of probes from the same container, constituting a second fraction of the total number of commingled probes, into the chamber of a second receptacle to form a second sensor comprised of at least one probe, wherein the second receptacle is different than and dissimilar to the first receptacle, (D) enclosing the chamber defined by the first receptacle, (E) enclosing the chamber defined by the second receptacle, (F) ascertaining target-analyte concentration within the enclosed chamber of the first receptacle by: (i) exposing the first sensor to excitation radiation to create an excited first sensor, (ii) measuring radiation emitted by the excited first sensor, and (iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm, and (G) ascertaining target-analyte concentration within the enclosed chamber of the second receptacle by: (i) exposing the second sensor to excitation radiation to create an excited second sensor, (ii) measuring radiation emitted by the excited second sensor, and (iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

[0013] A fifth aspect of the invention is a method for monitoring changes in target-analyte concentration within an enclosed space employing a target-analyte sensitive probe according to the first aspect of the invention. The method includes the steps of (A) obtaining a supply of the target-analyte sensitive probes according to the first aspect of the invention, (B) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe, (C) enclosing the space, (D) ascertaining target-analyte concentration within the enclosed space over time by: (i) taking at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor to excitation radiation to create an excited sensor, and (2) measuring radiation emitted by the excited sensor, (ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurement, and (iii) converting at least the identified emission measurements to a target-analyte concentration based upon a known conversion algorithm, and (E) reporting at least one of (i) at least the two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte concentration within the enclosed space calculated from data obtained in step (D).

[0014] A sixth aspect of the invention is a method for measuring concentration of a target-analyte of interest within an enclosed space employing different target-analyte sensitive probes according to the first aspect of the invention, wherein the target-analyte of interest is selectable from and selectively transitional amongst a plurality of different target-analytes without accessing the space once enclosed. The method includes the steps of (A) obtaining a supply of the target-analyte sensitive probes according to the first aspect of the invention wherein the supply includes probes that are sensitive to different target-analytes and uniquely interrogatable relative to one another, (B) placing at least two of the obtained probes sensitive to different target-analytes within a space, to form a sensor sensitive to at least two different target-analytes, (C) enclosing the space, and (D) ascertaining concentration of a target-analyte of interest within the enclosed space, selected from the at least two different target-analytes to which the at least two placed probes are sensitive, by: (i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the target-analyte of interest, (ii) measuring radiation emitted by the excited probe sensitive to the target-analyte of interest, and (iii) converting the measured emission to a concentration of the target-analyte of interest based upon a known conversion algorithm.

[0015] A seventh aspect of the invention is method for measuring concentration of at least two different target-analytes within an enclosed space employing different target-analyte sensitive probes according to the first aspect of the invention. The method includes the steps of (A) obtaining a first supply of target-analyte sensitive probes according to the first aspect of the invention which are sensitive to a first target-analyte, (B) obtaining a second supply of target-analyte sensitive probes according to the first aspect of the invention which are sensitive to a second target-analyte which is different from the first target-analyte, and wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another, (C) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space, to form a sensor sensitive to both first and second target-analytes, (D) enclosing the space, (E) ascertaining concentration of the first target-analyte within the enclosed space, by: (i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte, (ii) measuring radiation emitted by the excited probe sensitive to the first target-analyte, and (iii) converting the measured emission to a

concentration of the first target-analyte based upon a known conversion algorithm, and (F) ascertaining concentration of the second target-analyte within the enclosed space, by: (i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte, (ii) measuring radiation emitted by the excited probe sensitive to the second target-analyte, and (iii) converting the measured emission to a concentration of the second target-analyte based upon a known conversion algorithm.

[0016] An eighth aspect of the invention is method for monitoring changes in concentration of at least two different target-analytes within an enclosed space employing different target-analyte sensitive probes according to the first aspect of the invention. The method includes the steps of (A) obtaining a first supply of target-analyte sensitive probes according to the first aspect of the invention which are sensitive to a first target-analyte, (B) obtaining a second supply of target-analyte sensitive probes according to the first aspect of the invention which are sensitive to a second target-analyte which is different from the first target-analyte, and wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another, (C) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space, to form a sensor sensitive to both first and second target-analytes, (D) enclosing the space, (E) ascertaining concentration of the first target-analyte within the enclosed space, by: (i) taking a first set of at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte, and (2) measuring radiation emitted by the excited probe sensitive to the first target-analyte, (ii) measuring passage of time between at least two of the first set emission measurements to determine a time interval between identified first set emission measurements, and (iii) converting the measured emission to a concentration of the first target-analyte based upon a known conversion algorithm, and (F) ascertaining concentration of the second target-analyte within the enclosed space, by: (i) taking a second set of at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte, and (2) measuring radiation emitted by the excited probe sensitive to the second target-analyte, (ii) measuring passage of time between at least two of the second set emission measurements to determine a time interval between identified second set emission measurements, and (iii)

converting at least the identified second set emission measurements to a concentration of the second target-analyte based upon a known conversion algorithm, (G) reporting at least one of (i) at least two ascertained first target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in first target-analyte concentration within the enclosed space calculated from data obtained in step (E), and (H) reporting at least one of (i) at least two ascertained second target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in second target-analyte concentration within the enclosed space calculated from data obtained in step (F).

[0017] A ninth aspect of the invention is a method of manufacturing a probe according to the first aspect of the invention. The method includes the steps of (A) preparing a coating cocktail which contains at least the optically-active target-analyte sensitive material dissolved in a solvent, (B) applying the cocktail to the porous scaffold particle, and (C) allowing the applied cocktail to dry, whereby a solid-state thin film coating of optically-active target-analyte sensitive material is formed on the scaffold particle to form the probe.

[0018] In one embodiment of the invention there is provided a flexibly deployable, discrete target-analyte sensitive probe, comprising a porous scaffold particle coated with an optically-active, target-analyte sensitive material, wherein the scaffold particle has at least one of (i) a volume of 0.5 to 500 mm^3, and (ii) a largest dimension of 2 to 20 mm.

[0019] In one embodiment, the scaffold particle has a volume of 5 to 100 mm^3.

[0020] In one embodiment, the scaffold particle has a largest dimension of 3 to 10 mm.

[0021] In one embodiment, the probe has a density of greater than 1.2 g/cm^3.

[0022] In one embodiment, the scaffold particle material is porous glass.

[0023] In one embodiment, the scaffold particle is translucent to interrogation light.

[0024] In one embodiment, the target-analyte sensitive material is a photoluminescent material.

[0025] Preferably, the photoluminescent material includes at least a fluorescent or phosphorescent indicator dye having a responsive optical characteristic that changes in response to changes in the concentration or partial pressure of target-analyte to which the dye is exposed.

[0026] In one embodiment, (i) the responsive optical characteristic is at least one of photoluminescence lifetime and photoluminescence intensity, and (ii) the indicator dye is sensitive to the partial pressure of oxygen.

[0027] In one embodiment, the coating comprises a target-analyte sensitive indicator dye incorporated in a target-analyte permeable polymeric matrix.

[0028] Preferably, the target-analyte sensitive indicator dye is an oxygen sensitive photoluminescent transition metal complex selected from the group consisting of a ruthenium bipyridyl, a ruthenium diphenylphenanotroline, a platinum porphyrin, a palladium porphyrin, a phosphorescent complex of a tetrabenzoporphyrin, a chlorin, a porphyrin-ketone, an aza-porphyrin and a long-decay luminescent complex of iridium(III) or osmium(II).

[0029] Preferably, the polymeric matrix component is selected from the group consisting of silicone, polystrene, polycarbonate, and polysulfone.

[0030] In one embodiment of the invention, there is provided an article of commerce, comprising a plurality of probes as described above retained as a commingled supply of probes within a container from which the probes may be individually and discretely dispensed.

[0031] In one embodiment concerning the article of commerce, the probes within the container are sanitized probes.

[0032] In one embodiment concerning the article of commerce, the probes within the container are sterilized probes.

[0033] In one embodiment of the invention, there is provided a method for measuring concentration of a target-analyte within an enclosed space, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes as described above,

(b) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe,

(c) enclosing the space, and

(d) ascertaining target-analyte concentration within the enclosed space by:

(i) exposing the sensor to excitation radiation to create an excited sensor,

(ii) measuring radiation emitted by the excited sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

[0034] In one embodiment, the space is hermetically enclosed, and the method further comprises the step of placing a liquid test sample into the space prior to hermetically enclosing the space.

[0035] Preferably, (i) the space is enclosed within a receptacle, (ii) a single probe is placed within the space, and (ii) the probe is contactlessly interrogated through the receptacle.

[0036] More preferably, (i) the space is enclosed within a receptacle having a bottom, (ii) the method further comprises the step of placing a liquid test sample into the space prior to enclosing the space, (iii) the placed probes each have a density greater than the liquid test sample placed into the space, whereby the placed probes sink to the bottom of the receptacle, and (iv) the placed probes are contactlessly interrogated through the bottom of the receptacle.

[0037] In one embodiment of the invention, there is provided a method for measuring concentration of a target-analyte within an enclosed space, comprising the steps of:

(a) obtaining an article of commerce as described above,

(b) dispensing a known number of probes from the container, constituting a fraction of the total number of commingled probes, into a space to form a sensor comprised of at least one probe,

(c) enclosing the space, and

(d) ascertaining target-analyte concentration within the enclosed space by:

(i) exposing the sensor to excitation radiation to create an excited sensor,

(ii) measuring radiation emitted by the excited sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

[0038] In one embodiment of the invention, there is provided a method for measuring concentration of a target-analyte within the chamber of a plurality of receptacles, comprising the steps of:

(a) obtaining an article of commerce as described above,

(b) dispensing a first known number of probes from the container, constituting a first fraction of the total number of commingled probes, into a chamber defined by a first receptacle to form a first sensor comprised of at least one probe,

(c) dispensing a second known number of probes from the same container, constituting a second fraction of the total number of commingled probes, into the chamber of a second receptacle to form a second sensor comprised of at least one probe, wherein the second receptacle is different than and dissimilar to the first receptacle,

(d) enclosing the chamber defined by the first receptacle,

(e) enclosing the chamber defined by the second receptacle,

(f) ascertaining target-analyte concentration within the enclosed chamber of the first receptacle by:

(i) exposing the first sensor to excitation radiation to create an excited first sensor,

(ii) measuring radiation emitted by the excited first sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm, and

(g) ascertaining target-analyte concentration within the enclosed chamber of the second receptacle by:

(i) exposing the second sensor to excitation radiation to create an excited second sensor,

(ii) measuring radiation emitted by the excited second sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

[0039] In one embodiment of the invention, there is provided a method for monitoring changes in target-analyte concentration within an enclosed space, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes as described above,

(b) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe,

(c) enclosing the space,

(d) ascertaining target-analyte concentration within the enclosed space over time by:

(i) taking at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation to create an excited sensor, and

(2) measuring radiation emitted by the excited sensor,

(ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurement, and

(iii) converting at least the identified emission measurements to a target-analyte concentration based upon a known conversion algorithm, and

(e) reporting at least one of (i) at least the two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte concentration within the enclosed space calculated from data obtained in step (d).

[0040] In one embodiment, the space is hermetically enclosed, and the method further comprises the step of placing a liquid test sample into the space prior to hermetically enclosing the space.

[0041] In one embodiment, (i) the space is enclosed within a receptacle, (ii) a single probe is placed within the space, and (ii) the probe is contactlessly interrogated through the receptacle.

[0042] Preferably, the method is applied to achieve at least one of (i) a measurement of chemical activity of the test sample, (ii) a measurement of biological activity of the test sample, (iii) a presence/absence determination of a threshold concentration of aerobic

microorganisms in the test sample, and (iv) an enumeration of aerobic microorganisms in the test sample at the time the test sample is placed in the space.

[0043] In one embodiment of the invention, there is provided a method for monitoring changes in target-analyte concentration within an enclosed space, comprising the steps of:

(a) obtaining an article of commerce as described above,

(b) dispensing a known number of probes from the container, constituting a fraction of the total number of commingled probes, into a space to form a sensor comprised of at least one probe,

(c) enclosing the space,

(d) ascertaining target-analyte concentration within the enclosed space over time by:

(i) taking at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation to create an excited sensor, and

(2) measuring radiation emitted by the excited sensor,

(ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurement, and

(iii) converting at least the identified emission measurements to a target-analyte concentration based upon a known conversion algorithm, and

(e) reporting at least one of (i) at least two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte concentration within the enclosed space calculated from data obtained in step (d).

[0044] In one embodiment of the invention, there is provided a method for measuring concentration of a target-analyte of interest within an enclosed space wherein the target-analyte of interest is selectable from and selectively transitional amongst a plurality of different target-analytes without accessing the space once enclosed, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes as described above wherein the supply includes probes that are sensitive to different target-analytes and uniquely interrogatable relative to one another,

(b) placing at least two of the obtained probes sensitive to different target-analytes within a space, to form a sensor sensitive to at least two different target-analytes,

(c) enclosing the space, and

(d) ascertaining concentration of a target-analyte of interest within the enclosed space, selected from the at least two different target-analytes to which the at least two placed probes are sensitive, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the target-analyte of interest,

(ii) measuring radiation emitted by the excited probe sensitive to the target-analyte of interest, and

(iii) converting the measured emission to a concentration of the target-analyte of interest based upon a known conversion algorithm.

[0045] In one embodiment of the invention, there is provided a method for measuring concentration of at least two different target-analytes within an enclosed space, comprising the steps of:

(a) obtaining a first supply of target-analyte sensitive probes as described above sensitive to a first target-analyte,

(b) obtaining a second supply of target-analyte sensitive probes as described above sensitive to a second target-analyte which is different from the first target-analyte, wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another,

(c) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space, to form a sensor sensitive to both first and second target-analytes,

(d) enclosing the space,

(e) ascertaining concentration of the first target-analyte within the enclosed space, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte,

(ii) measuring radiation emitted by the excited probe sensitive to the first target-analyte, and

(iii) converting the measured emission to a concentration of the first target-analyte based upon a known conversion algorithm, and

(f) ascertaining concentration of the second target-analyte within the enclosed space, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte,

(ii) measuring radiation emitted by the excited probe sensitive to the second target-analyte, and

(iii) converting the measured emission to a concentration of the second target-analyte based upon a known conversion algorithm.

[0046] In one embodiment of the invention, there is provided a method for monitoring changes in concentration of at least two different target-analytes within an enclosed space, comprising the steps of:

(a) obtaining a first supply of target-analyte sensitive probes as described above sensitive to a first target-analyte,

(b) obtaining a second supply of target-analyte sensitive probes as described above sensitive to a second target-analyte which is different from the first target-analyte, wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another,

(c) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space to form a sensor sensitive to both first and second target-analytes,

(d) enclosing the space,

(e) ascertaining concentration of the first target-analyte within the enclosed space over time by:

(i) taking a first set of at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte, and

(2) measuring radiation emitted by the excited probe sensitive to the first target-analyte,

(ii) measuring passage of time between at least two of the first set emission measurements to determine a time interval between identified first set emission measurements, and

(iii) converting at least the identified first set emission measurements to a concentration of the first target-analyte based upon a known conversion algorithm,

(f) ascertaining concentration of the second target-analyte within the enclosed space over time by:

(i) taking a second set of at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte, and

(2) measuring radiation emitted by the excited probe sensitive to the second target-analyte,

(ii) measuring passage of time between at least two of the second set emission measurements to determine a time interval between identified second set emission measurements, and

(iii) converting at least the identified second set emission measurements to a concentration of the second target-analyte based upon a known conversion algorithm,

(g) reporting at least one of (i) at least two ascertained first target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in first target-analyte concentration within the enclosed space calculated from data obtained in step (e), and

(h) reporting at least one of (i) at least two ascertained second target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in second target-analyte concentration within the enclosed space calculated from data obtained in step (f).

[0047] In one embodiment of the invention, there is provided a method of preparing the probe according to the invention, which includes at least the steps of:

(a) preparing a coating cocktail which contains at least the optically-active target-analyte sensitive material dissolved in a solvent,

(b) applying the cocktail onto the porous scaffold particle, and

(c) allowing the applied cocktail to dry, whereby a solid-state thin film coating of optically-active target-analyte sensitive material is formed on the scaffold particle to form the probe.

[0048] In one embodiment, the coating cocktail further includes a polymer operable for forming a target-analyte permeable polymer matrix when dried.

[0049] In one embodiment, the scaffold particle is soaked with the cocktail whereby the cocktail penetrates into and coats the pores in the scaffold particle, and the method further includes separating the coated scaffold particle from excess cocktail prior to drying.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] Figure 1 is an enlarged, cross-sectional side-view of one embodiment of a probe according to the invention wherein the coated pores in the scaffold particle are not depicted and the coating of optically-active, target-analyte sensitive material is exaggerated to facilitate depiction of the coating.

[0051] Figure 2 is a grossly enlarged, cross-sectional side view of a surface portion of the probe depicted in Figure 1.

[0052] Figure 3 is a schematic drawing of probes according to the invention employed to measure target-analyte in an assay vessel testing receptacle.

[0053] Figure 4 is a schematic drawing probes according to the invention employed to measure target-analyte in multiple assay vessel testing receptacles.

[0054] Figure 5 is a schematic drawing of probes according to the invention, sorted by the target-analyte to which they are sensitive, employed to measure different target-analytes within the same assay vessel testing receptacle.

[0055] Figure 6 is a top view of an alternative collated packaging of the sorted probes depicted in Figure 5.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Definitions

[0056] As used herein, including the claims, the term "***target-analyte***" refers to a chemical substance, typically O_2, CO_2 or pH, capable of proportionally altering an optical property of an optically-active material containing a photoluminescent dye.

[0057] As used herein, including the claims, the term "***contactless interrogation***", means interrogation without tangible physical contact with the interrogated device, whereby interrogation can occur through an intervening barrier.

[0058] As used herein, including the claims, the term "***interrogation light***" means electromagnetic radiation having a wavelength between 400 and 900 nm.

Nomenclature

10 Probe

10_{A1} Probe Sensitive to Target Analyte A1

10_{A2} Probe Sensitive to Target Analyte A2

20 Scaffold Particle

29 Pores in Scaffold Particle

30 Target-Analyte Sensitive Coating

31 Target-Analyte-Sensitive Photoluminescent Dye

32 Target-Analyte-Permeable Polymer Matrix

40 Container for Supply of Probes

50 Assay Vessel

50_1 First Assay Vessel

50_2 Second Assay Vessel

50a Open Top of Assay Vessel

50b Bottom of Assay Vessel

59 Chamber of Assay Vessel

100 Supply of Probes

100_1 First Supply of Probes

100_2 Second Supply of Probes

200 Sensor

200_1 First Sensor

200_2 Second Sensor

A Target-Analyte

A1 First Target-Analyte

A2 Second Target-Analyte

S Test Sample

Description

Theory

[0059] The methods and compositions described herein are based on the quenching of an optical property, typically photoluminescence, by a target-analyte, typically oxygen (O_2). Luminescence encompasses both fluorescence and phosphorescence. Electromagnetic radiation in the ultraviolet or visible region is used to excite molecules to higher electronic energy levels. The excited molecules lose their excess energy by one of several methods. One of those methods is fluorescence. Fluorescence refers to the radiative transition of electrons from the first excited singlet state to the singlet ground state (S_1 .to S_0). The lifetime of fluorescence is relatively short, approximately 10^{-9} to 10^{-7} seconds. However, intersystem crossing from the lowest excited singlet state to the triplet state often occurs and is attributed to the crossing of the potential energy curves of the two states. The triplet state so produced may return to the ground state by a radiative process known as phosphorescence. Phosphorescence is the radiative relaxation of an electron from the lowest excited triplet state to the singlet ground state (T_1 to S_0). Because the transition that leads to phosphorescence involves a change in spin multiplicity, it has a low probability and hence a relatively long lifetime of 10^{-4} to 10 seconds. Fluorescent and phosphorescent intensity and lifetime are known to change in a defined fashion relative to changes in the partial pressure of a target-analyte capable of quenching the photoluminescent molecules. Hence, the partial pressure of a target-analyte in fluid communication with a photoluminescent material can be determined by measuring photoluminescence intensity and/or lifetime.

Construction

[0060] A first aspect of the invention are probes **10** capable of reporting the partial pressure, and thereby the concentration, of a target-analyte **A** (P_A). The probes **10** are inexpensive, discrete, self-contained particles, which are remotely interrogatable by optical means and autonomously positionable, thereby permitting the probes **10** to be used for a wide variety of purposes and in combination with a wide variety of assay vessels to quickly, easily and reliably measure and monitor changes in analyte concentration in an environment. The

probes **10** are particularly well suited for measuring and monitoring changes in target-analyte concentration in an enclosed environment in a non-invasive and non-destructive manner.

[0061] The probes **10** are sensitive to a target-analyte **A**, such as O_2, CO_2, CO or H^+. For purposes of simplicity only, and without intending to be limited thereto, the balance of the description shall reference O_2 as the target-analyte **A** since O_2-sensitive probes are the most commonly used types of optically active probes.

[0062] Referring to Figures 1 and 2, the probes **10** each comprise a suitably sized porous scaffold particle **20** coated with a target-analyte **A** sensitive coating **30**.

[0063] One of routine skill in the art is capable of selecting a suitable microporous scaffold particle **20** based upon the intended use of the probe **10**, the target-analyte **A** of interest and the composition of the target-analyte sensitive coating **30**. The porous scaffold particle **20** is preferably structurally stable, compatible with the solvent based target-analyte sensitive coating **30** during the coating process, and the dry coating **30**, inert when used in accordance with its intended use, and exhibits excellent light scattering properties at the excitation and emission wavelengths for the target-analyte **A** sensitive coating **30**. A nonexhaustive list of suitable materials for use as the porous scaffold particle **20** includes specifically, but not exclusively, glass, and polymers such as polyethylene, polypropylene, polytetrafluoroethylene, polystryrene, polycarbonate, polysulfone, polyvinyl chloride, cross-linked poly(styrene-divinylbenzene) and other similar co-polymers. Based upon its superior structural stability, coating compatability, and density, the preferred material is glass. The scaffold particle **20** is preferably constructed from a material which is translucent to interrogation light. The scaffold particle **20** is preferably a non-metalic, most preferably a nonferrous material, due to weight, cost and inability to provide sufficient porosity.

[0064] The scaffold particles **20** are preferably dry, homogeneous and non-aggregating. They may be in the form of beads, fibers, filaments, fines, pellets, powder, prills and the like.

[0065] The scaffold particles **20** are preferably selected so that the resultant particulate probe **10** has a density of greater than 1.2 g/cm^3, most preferably a density of between 1.5 and 5 g/cm^3 so that the probe **10**, when introduced into the chamber **59** of an assay vessel **50** through the open top **50a** of the vessel **50** will remain at or sink to the bottom **50b** of the vessel **50** when combined with common test sample materials **S**, thereby facilitating location and interrogation of the probe **10** within the assay vessel **50**.

[0066] The target-analyte sensitive coating **30** is preferrably includes a target-analyte-sensitive photoluminescent dye **31** embedded with a target-analyte **A** permeable polymer matrix **32**.

[0067] One of routine skill in the art is capable of selecting a suitable indicator dye **31** based upon the target-analyte **A** of interest and the intended use of the probe **10**. Preferred photoluminescent indicator dyes **31** are long-decay fluorescent or phosphorescent indicator dyes. For example, a nonexhaustive list of suitable P_{O2} sensitive photoluminescent indicator dyes **31** includes specifically, but not exclusively, ruthenium(II)-bipyridyl and ruthenium(II)-diphenylphenanothroline complexes, porphyrin-ketones such as platinum(II)-octaethylporphine-ketone, platinum(II)-porphyrin such as platinum(II)-tetrakis(pentafluorophenyl)porphine, palladium(II)-porphyrin such as palladium(II)-tetrakis(pentafluorophenyl)porphine, phosphorescent metallocomplexes of tetrabenzoporphyrins, chlorins, azaporphyrins, and long-decay luminescent complexes of iridium(III) or osmium(II).

[0068] Typically and preferably, the target-analyte sensitive photoluminescent dye **31** is compounded with and embedded within a suitable target-analyte permeable polymer matrix **32**. Again, one of routine skill in the art is capable of selecting a suitable polymeric matrix **32** based upon the target-analyte **A** of interest, the selected dye **31** and the intended use of the probe **10**. For example, a nonexhaustive list of suitable polymers for use as the oxygen-permeable polymer matrix **32** includes specifically, but not exclusively, polystryrene, polycarbonate, polysulfone, polyvinyl chloride and some co-polymers.

[0069] The optically active particulate probe **10** preferably has at least one of a volume between 0.5 and 500 mm^3, preferably 5 to 100 mm^3, and a largest dimension of between 2 and **20** mm, preferably 3 to 10 mm. Probes **10** with a volume smaller than 0.5 mm^3 and/or a largest dimension of less than 2 mm are difficult to handle and accurately dispense, while probes **10** with a volume greater than 500 mm^3 and/or a largest dimension of greater than **20** mm are too large for use in many common testing receptacles **50**, such as many traditional assay vessels, and unnecessarily increases the cost of each probe **10**.

Manufacture and Supply

[0070] The optically active particulate probe **10** can be manufactured by any suitable technique. It is generally advantageous for the scaffold particle **20** to have a uniform size.

The particle **20** may have a geometrical (*e.g.*, planar, rectangular, spherical, oval, etc) or irregular shape and may have a rough or smooth exterior surface.

[0071] One technique is to dissolve or suspend the indicator dye **31**, preferably with target-analyte permeable polymer **32**, in a suitable organic solvent such as ethylacetate, immersing the porous scaffold particles **20** of the desired type, size and shape in the solution to coat the particles **20**, including the pores **29**, with dye **31**, removing the coated particles **20**, and allowing the coated particles **20** to dry. Alternatively, the solution may be sprayed onto the particles **20**. Generally, the concentration of indicator dye **21** in the organic solvent should be in the range of 0.01 to 5% w/w. Generally, the concentration of the polymer **32** in the organic solvent should be in the range of 0.1 to 20% w/w, with the ratio of indicator dye **31** to polymer **32** in the range of 1:50 to 1:5,000 w/w.

[0072] The probes **10** can be supplied as an article of commerce comprising a plurality of the probes **10**, (*e.g.*, 10 to 1,000 probes or more) retained as a commingled supply **100** of the probes **10** within a container **40** from which the probes **10** may be individually and discretely dispensed. Referring to Figures 5 and 6, when different probes **10** sensitive to different target-analytes **A** (*e.g.*, probe $\mathbf{10_{A1}}$ sensitive to target-analyte **A1** and probe $\mathbf{10_{A2}}$ sensitive to target-analyte **A2**) are to be placed into a testing receptacle such as an assay vessel **50**, the supply $\mathbf{100_1}$ and $\mathbf{100_2}$ of each probe $\mathbf{10_{A1}}$ and $\mathbf{10_{A2}}$ may be sorted, as shown in Figure 5, or collated as shown in Figure 6.

[0073] The particulate probes **10** may be sanitized or sterilized before or after being deposited into the container **40** by any suitable means, such as heat, gamma irradiation or ethylene oxide, on order to avoid microbial contamination of a sample **S** undergoing microbial testing with the probes **10**.

Use

Measuring Concentration of Single Target-Analyte A

[0074] Referring generally to Figures 3-5, the probe **10** can be used to quickly, easily, accurately and reliably measure the concentration of a target-analyte **A** in an environment (*e.g.*, the sealed chamber **59** of an assay vessel **50** or the sealed chamber (not shown) of a package (not shown) containing a product (not shown) susceptible to spoilage or deterioration). The probe **10** can be interrogated in the same manner as typical target-analyte **A** sensitive photoluminescent probes are interrogated. Briefly, the probe **10** is used to

measure the concentration of a target-analyte **A** in an environment by (A) placing the probe **10** into fluid communication with the environment to be monitored (*e.g.*, within the sealed chamber **59** of an assay vessel **50** containing a test sample **S**) at a location where radiation at the excitation and emission wavelengths of the indicator dye **31** can be transmitted to and received from the probe **10** with minimal interference and without opening or otherwise breaching the integrity of the environment (*e.g.*, without opening the assay vessel **50**), (B) interrogating the probe **10** with an interrogation device (not shown), and (C) converting the measured emissions to a target-analyte **A** concentration within the environment based upon a known conversion algorithm or look-up table.

[0075] The radiation emitted by the excited probe **10** can be measured in terms of photoluminescence intensity, intensity ratio and/or lifetime (rate of decay, phase shift or anisotropy), with measurement of lifetime generally preferred as a more accurate and reliable measurement technique when seeking to establish the extent to which the indicator dye **31** has been quenched by target-analyte **A**.

[0076] Referring to Figure 3, a preferred method of measuring the concentration of a target-analyte A within an enclosed space **59** employing a probe **10** includes the steps of (A) obtaining a supply **100** of the target-analyte sensitive probes **10**, (B) placing a known number of probes **10** from the supply **100** of probes **10** within a space **59** to form a sensor **200** comprised of at least one probe **10**, (C) enclosing the space **59**, and (D) ascertaining target-analyte **A** concentration within the enclosed space **59** by: (i) exposing the sensor **200** to excitation radiation to create an excited sensor **200**, (ii) measuring radiation emitted by the excited sensor **200**, and (iii) converting the measured emission to a target-analyte **A** concentration based upon a known conversion algorithm.

[0077] Probes **10** are uniquely suited for measuring concentration of a target-analyte A within the chamber **59** of a plurality of dissimilar assay vessels **50**. Referring to Figure 4, the method includes the steps of (A) obtaining a supply **100** of probes **10** retained within a container **40**, (B) dispensing a first known number of probes **10** from the container **40**, constituting a first fraction of the total number of commingled probes **10**, into a chamber **59** defined by a first assay vessel $\mathbf{50_1}$ to form a first sensor $\mathbf{200_1}$ comprised of at least one probe **10**, (C) dispensing a second known number of probes **10** from the same container **40**, constituting a second fraction of the total number of commingled probes **10**, into the chamber **59** of a second assay vessel $\mathbf{50_2}$ to form a second sensor $\mathbf{200_2}$ comprised of at least one probe **10**, wherein the second assay vessel $\mathbf{50_2}$ is different than and dissimilar to the first r assay

vessel **50_1**, (D) enclosing the chamber **59** defined by the first assay vessel **50_1**, (E) enclosing the chamber **59** defined by the second assay vessel **50_2**, (F) ascertaining target-analyte **A** concentration within the enclosed chamber **59** of the first assay vessel **50_1** by: (i) exposing the first sensor **200_1** to excitation radiation to create an excited first sensor **200_1**, (ii) measuring radiation emitted by the excited first sensor **200_1**, and (iii) converting the measured emission to a target-analyte **A** concentration based upon a known conversion algorithm, and (G) ascertaining target-analyte concentration within the enclosed chamber **59** of the second assay vessel **50_2** by: (i) exposing the second sensor **200_2** to excitation radiation to create an excited second sensor **200_2**, (ii) measuring radiation emitted by the excited second sensor **200_2**, and (iii) converting the measured emission to a target-analyte **A** concentration based upon a known conversion algorithm.

Monitoring Changes in Single Target-Analyte A Concentration

[0078] The probe **10** can also be used to quickly, easily, accurately and reliably monitor changes in target-analyte **A** concentration in an environment by (i) placing the probe **10** into fluid communication with the environment to be monitored at a location where radiation at the excitation and emission wavelengths of the indicator dye **31** can be transmitted to and received from the probe **10** with minimal interference and without opening or otherwise breaching the integrity of the environment, (B) ascertaining the target-analyte **A** concentration within the environment over time by (i) repeatedly exposing the probe **10** to excitation radiation over time, (ii) measuring radiation emitted by the excited probe **10** after at least some of the exposures, (iii) measuring passage of time during the repeated excitation exposures and emission measurements, and (iv) converting at least some of the measured emissions to a target-analyte **A** concentration based upon a known conversion algorithm, and (C) reporting at least one of (i) at least two ascertained target-analyte **A** concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte **A** concentration within the environment calculated from data obtained in step (B). Conversion algorithms used to convert the measured emissions to a target-analyte concentration are well know to and readily developable by those with routine skill in the art.

[0079] Referring to Figure 3, a preferred method for monitoring changes in target-analyte **A** concentration within an enclosed space **59** employing a probe **10** includes the steps of (A) obtaining a supply **100** of the probes **10**, (B) placing a known number of probes **10** from the supply **100** of probes **10** within a space **59** to form a sensor **200** comprised of at least one

probe **10**, (C) enclosing the space **59**, (D) ascertaining target-analyte **A** concentration within the enclosed space **59** over time by: (i) taking at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor **200** to excitation radiation to create an excited sensor **200**, and (2) measuring radiation emitted by the excited sensor **200**, (ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurements, and (iii) converting at least the identified emission measurements to a target-analyte A concentration based upon a known conversion algorithm, and (E) reporting at least one of (i) at least the two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte A concentration within the enclosed space **59** calculated from data obtained in step (D).

Measuring Concentration of Deferrably Selectable Target-Analyte A

[0080] Probes **10** are also uniquely suited for measuring concentration of a target-analyte A of interest within an enclosed space **59** employing different probes **10** sensitive to different target-analytes **A** (*e.g.*, probe $\mathbf{10}_{\mathbf{A1}}$ sensitive to target-analyte **A1** and probe $\mathbf{10}_{\mathbf{A2}}$ sensitive to target-analyte **A2**), wherein the target-analyte **A** of interest is selectable from and selectively transitional amongst a plurality of different target-analytes **A** without accessing the space **59** once enclosed. The method includes the steps of (A) obtaining a supply **100** of probes **10** wherein the supply includes probes **10** that are sensitive to different target-analytes **A** (*e.g.*, a supply $\mathbf{100}_1$ of probes $\mathbf{10}_{\mathbf{A1}}$ sensitive to target-analyte **A1** and a supply $\mathbf{100}_2$ of probes $\mathbf{10}_{\mathbf{A2}}$ sensitive to target-analyte **A2**) and are uniquely interrogatable relative to one another, (B) placing at least two of the obtained probes **10** sensitive to different target-analytes **A** within a space **59**, to form a sensor **200** sensitive to at least two different target-analytes **A** (*e.g.*, target-analyte **A1** and target-analyte **A2**), (C) enclosing the space **59**, and (D) ascertaining concentration of a target-analyte **A** of interest within the enclosed space, selected from the at least two different target-analytes **A** to which the at least two placed probes **10** are sensitive (*e.g.*, probe $\mathbf{10}_{\mathbf{A1}}$ sensitive to target-analyte **A1** and probe $\mathbf{10}_{\mathbf{A2}}$ sensitive to target-analyte **A2**), by: (i) exposing the sensor **200** to excitation radiation effective for exciting the probe **10** sensitive to the target-analyte **A** of interest (*e.g.*, probe $\mathbf{10}_{\mathbf{A1}}$ sensitive to target-analyte **A1**), (ii) measuring radiation emitted by the excited probe **10** sensitive to the target-analyte **A** of interest (*e.g.*, probe $\mathbf{10}_{\mathbf{A1}}$ sensitive to target-analyte **A1**), and (iii) converting the measured emission to a concentration of the target-analyte **A** of interest (*e.g.*, target-analyte **A1**) based upon a known conversion algorithm.

Measuring Concentration of Plurality of Different Target-Analytes A

[0081] Probes **10** are also uniquely suited for measuring the concentration of at least two different target-analytes A (*e.g.*, target-analyte **A1** and target-analyte **A2**) within an enclosed space **59** employing different target-analyte sensitive probes **10** (*e.g.*, probe $\mathbf{10_{A1}}$ sensitive to target-analyte **A1** and probe $\mathbf{10_{A2}}$ sensitive to target-analyte **A2**). The method includes the steps of (A) obtaining a first supply $\mathbf{100_1}$ of target-analyte sensitive probes $\mathbf{10_{A1}}$ which are sensitive to a first target-analyte **A1**, (B) obtaining a second supply $\mathbf{100_2}$ of target-analyte sensitive probes $\mathbf{10_{A2}}$ which are sensitive to a second target-analyte **A2** which is different from the first target-analyte **A1**, and wherein the probes $\mathbf{10_{A1}}$ sensitive to a first target-analyte **A1** and the probes $\mathbf{10_{A2}}$ sensitive to a second target-analyte **A2** are uniquely interrogatable relative to one another, (C) placing at least one probe $\mathbf{10_{A1}}$ from the first supply of probes $\mathbf{100_1}$ and at least one probe $\mathbf{10_{A2}}$ from the second supply of probes $\mathbf{100_2}$ within a space **50**, to form a sensor **200** sensitive to both first **A1** and second **A2** target-analytes, (D) enclosing the space **59**, (E) ascertaining concentration of the first target-analyte **A1** within the enclosed space **59**, by: (i) exposing the sensor **200** to excitation radiation effective for exciting the probe $\mathbf{10_{A1}}$ sensitive to the first target-analyte **A1**, (ii) measuring radiation emitted by the excited probe $\mathbf{10_{A1}}$ sensitive to the first target-analyte **A1**, and (iii) converting the measured emission to a concentration of the first target-analyte **A1** based upon a known conversion algorithm, and (F) ascertaining concentration of the second target-analyte **A2** within the enclosed space **59**, by: (i) exposing the sensor **200** to excitation radiation effective for exciting the probe $\mathbf{10_{A2}}$ sensitive to the second target-analyte **A2**, (ii) measuring radiation emitted by the excited probe $\mathbf{10_{A2}}$ sensitive to the second target-analyte **A2**, and (iii) converting the measured emission to a concentration of the second target-analyte **A2** based upon a known conversion algorithm.

Monitoring Changes in Concentration of Multiple Target-Analytes A

[0082] Probes **10** are suited for simultaneously monitoring changes in the concentration of different target-analytes **A** of interest (*e.g.*, target-analyte **A1** and target-analyte **A2**) within an enclosed space **59**. The method includes the steps of (A) obtaining a first supply $\mathbf{100_1}$ of target-analyte sensitive probes $\mathbf{10_{A1}}$ which are sensitive to a first target-analyte **A1**, (B) obtaining a second supply $\mathbf{100_2}$ of target-analyte sensitive probes $\mathbf{10_{A2}}$ which are sensitive to a second target-analyte **A2** which is different from the first target-analyte **A1**, and wherein the probes $\mathbf{10_{A1}}$ sensitive to a first target-analyte **A1** and the probes $\mathbf{10_{A2}}$ sensitive to a second

target-analyte **A2** are uniquely interrogatable relative to one another, (C) placing at least one probe $\mathbf{10}_{A1}$ from the first supply of probes $\mathbf{100}_1$ and at least one probe $\mathbf{10}_{A2}$ from the second supply of probes $\mathbf{100}_2$ within a space **59**, to form a sensor **200** sensitive to both first **A1** and second **A2** target-analytes, (D) enclosing the space **59**, (E) ascertaining concentration of the first target-analyte **A1** within the enclosed space **59**, by: (i) taking a first set of at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor **200** to excitation radiation effective for exciting the probe $\mathbf{10}_{A1}$ sensitive to the first target-analyte, **A1** and (2) measuring radiation emitted by the excited probe $\mathbf{10}_{A1}$ sensitive to the first target-analyte **A1**, (ii) measuring passage of time between at least two of the first set emission measurements to determine a time interval between identified first set emission measurements, and (iii) converting the measured emission to a concentration of the first target-analyte based upon a known conversion algorithm, and (F) ascertaining concentration of the second target-analyte **A2** within the enclosed space **59**, by: (i) taking a second set of at least two emission measurements over time, each measurement comprising the steps of: (1) exposing the sensor **200** to excitation radiation effective for exciting the probe $\mathbf{10}_{A2}$ sensitive to the second target-analyte **A2**, and (2) measuring radiation emitted by the excited probe $\mathbf{10}_{A2}$ sensitive to the second target-analyte **A2**, (ii) measuring passage of time between at least two of the second set emission measurements to determine a time interval between identified second set emission measurements, and (iii) converting at least the identified second set emission measurements to a concentration of the second target-analyte based upon a known conversion algorithm, (G) reporting at least one of (i) at least two ascertained first target-analyte **A1** concentrations and the time interval between those reported concentrations, and (ii) a rate of change in first target-analyte **A1** concentration within the enclosed space **59** calculated from data obtained in step (E), and (H) reporting at least one of (i) at least two ascertained second target-analyte **A2** concentrations and the time interval between those reported concentrations, and (ii) a rate of change in second target-analyte **A2** concentration within the enclosed space **59** calculated from data obtained in step (F).

EXAMPLES

Example 1

(Manufacture of O_2 Particulate Probes)

[0083] The phosphorescent oxygen-sensitive dye platinum(II) benzoporphyrin was dissolved in a 5% solution of polystyrene in ethylacetate at a concentration of 1 mg platinum(II) benzoporphyrin / ml of solution to form a coating cocktail. 100g of 4 mm diameter porous glass beads were soaked in the cocktail, separated on a mesh screen, dried on aluminum foil at room temperature for 2 hours, and placed into a container for future use.

Example 2.

(Use of O_2 Particulate Probes To Detect Sample Sterility)

[0084] The probe produced according to Example 1 is transferred aseptically into a 15 ml sterile plastic vial with a screw cap. An aliquot of food or a medical sample, combined with a medium for supporting growth of microorganisms, is added aseptically to the vial along with the probe. The vial is capped and immediately thereafter the probe within the vial is externally interrogated with an Optech™ platinum external detector available from Mocon, Inc. to obtain an initial t_0 photoluminescence lifetime signal. The vial with its contents is incubated in an incubator at 30°C for 24 hours, at which time the probe within the vial is again interrogated with the Optech™ platinum detector and an incubated t_1 photoluminescence lifetime signal measured. Any increase in the signal after incubation ($t_1 - t_0$) indicates a proportional decrease in O_2 concentration within the vial as a result of the metabolic consumption of O_2 within the vial by microorganism introduced into the vial by the sample aliquot. A significant increase denotes that the sample aliquot as introduced into the vial was non-sterile, while a lack of any increase or a small increase denotes that the sample aliquot as introduced into the vial was sterile.

We claim:

1. A flexibly deployable, discrete target-analyte sensitive probe, comprising a porous scaffold particle coated with an optically-active, target-analyte sensitive material, wherein the scaffold particle has at least one of (i) a volume of 0.5 to 500 mm^3, and (ii) a largest dimension of 2 to 20 mm.

2. The probe of claim 1 wherein the scaffold particle has a volume of 5 to 100 mm^3.

3. The probe of claim 1 wherein the scaffold particle has a largest dimension of 3 to 10 mm.

4. The probe of claim 1 wherein the probe has a density of greater than 1.2 g/cm^3.

5. The probe of claim 1 wherein the scaffold particle material is porous glass.

6. The probe of claim 1 wherein the scaffold particle is translucent to interrogation light.

7. The probe of claim 1 wherein the target-analyte sensitive material is a photoluminescent material.

8. The probe of claim 7 wherein the photoluminescent material includes at least a fluorescent or phosphorescent indicator dye having a responsive optical characteristic that changes in response to changes in the concentration or partial pressure of target-analyte to which the dye is exposed.

9. The probe of claim 8 wherein (i) the responsive optical characteristic is at least one of photoluminescence lifetime and photoluminescence intensity, and (ii) the indicator dye is sensitive to the partial pressure of oxygen.

10. The probe of claim 1 wherein the coating comprises a target-analyte sensitive indicator dye incorporated in a target-analyte permeable polymeric matrix.

11. The probe of claim 10 wherein the target-analyte sensitive indicator dye is an oxygen sensitive photoluminescent transition metal complex selected from the group consisting of a ruthenium bipyridyl, a ruthenium diphenylphenanotroline, a platinum porphyrin, a palladium porphyrin, a phosphorescent complex of a tetrabenzoporphyrin, a chlorin, a porphyrin-ketone, an aza-porphyrin and a long-decay luminescent complex of iridium(III) or osmium(II).

12. The probe of claim 10 wherein the polymeric matrix component is selected from the group consisting of silicone, polystrene, polycarbonate, and polysulfone.

13. An article of commerce, comprising a plurality of probes in accordance with any of claims 1-12 retained as a commingled supply of probes within a container from which the probes may be individually and discretely dispensed.

14. The article of commerce of claim 13 wherein the probes within the container are sanitized probes.

15. The article of commerce of claim 13 wherein the probes within the container are sterilized probes.

16. A method for measuring concentration of a target-analyte within an enclosed space, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes according to any one of claims 1-12,

(b) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe,

(c) enclosing the space, and

(d) ascertaining target-analyte concentration within the enclosed space by:

(i) exposing the sensor to excitation radiation to create an excited sensor,

(ii) measuring radiation emitted by the excited sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

17. The method of claim 16 wherein the space is hermetically enclosed, and the method further comprises the step of placing a liquid test sample into the space prior to hermetically enclosing the space.

18. The method of claim 16 wherein (i) the space is enclosed within a receptacle, (ii) a single probe is placed within the space, and (ii) the probe is contactlessly interrogated through the receptacle.

19. The method of claim 16 wherein (i) the space is enclosed within a receptacle having a bottom, (ii) the method further comprises the step of placing a liquid test sample into the space prior to enclosing the space, (iii) the placed probes each have a density greater than the liquid test sample placed into the space, whereby the placed probes sink to the bottom of the receptacle, and (iv) the placed probes are contactlessly interrogated through the bottom of the receptacle.

20. A method for measuring concentration of a target-analyte within an enclosed space, comprising the steps of:

(a) obtaining an article of commerce according to any one of claims 13-15,

(b) dispensing a known number of probes from the container, constituting a fraction of the total number of commingled probes, into a space to form a sensor comprised of at least one probe,

(c) enclosing the space, and

(d) ascertaining target-analyte concentration within the enclosed space by:

(i) exposing the sensor to excitation radiation to create an excited sensor,

(ii) measuring radiation emitted by the excited sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

21. A method for measuring concentration of a target-analyte within the chamber of a plurality of receptacles, comprising the steps of:

(a) obtaining an article of commerce according to any one of claims 13-15,,

(b) dispensing a first known number of probes from the container, constituting a first fraction of the total number of commingled probes, into a chamber

defined by a first receptacle to form a first sensor comprised of at least one probe,

(c) dispensing a second known number of probes from the same container, constituting a second fraction of the total number of commingled probes, into the chamber of a second receptacle to form a second sensor comprised of at least one probe, wherein the second receptacle is different than and dissimilar to the first receptacle,

(d) enclosing the chamber defined by the first receptacle,

(e) enclosing the chamber defined by the second receptacle,

(f) ascertaining target-analyte concentration within the enclosed chamber of the first receptacle by:

(i) exposing the first sensor to excitation radiation to create an excited first sensor,

(ii) measuring radiation emitted by the excited first sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm, and

(g) ascertaining target-analyte concentration within the enclosed chamber of the second receptacle by:

(i) exposing the second sensor to excitation radiation to create an excited second sensor,

(ii) measuring radiation emitted by the excited second sensor, and

(iii) converting the measured emission to a target-analyte concentration based upon a known conversion algorithm.

22. A method for monitoring changes in target-analyte concentration within an enclosed space, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes according to any one of claims 1-12,

(b) placing a known number of probes from the supply of probes within a space to form a sensor comprised of at least one probe,

(c) enclosing the space,

(d) ascertaining target-analyte concentration within the enclosed space over time by:

(i) taking at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation to create an excited sensor, and

(2) measuring radiation emitted by the excited sensor,

(ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurement, and

(iii) converting at least the identified emission measurements to a target-analyte concentration based upon a known conversion algorithm, and

(e) reporting at least one of (i) at least the two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte concentration within the enclosed space calculated from data obtained in step (d).

23. The method of claim 22 wherein the space is hermetically enclosed, and the method further comprises the step of placing a liquid test sample into the space prior to hermetically enclosing the space.

24. The method of claim 22 wherein (i) the space is enclosed within a receptacle, (ii) a single probe is placed within the space, and (ii) the probe is contactlessly interrogated through the receptacle.

25. The method of claim 23 wherein the method is applied to achieve at least one of (i) a measurement of chemical activity of the test sample, (ii) a measurement of biological activity of the test sample, (iii) a presence/absence determination of a threshold concentration of aerobic microorganisms in the test sample, and (iv) an enumeration of aerobic microorganisms in the test sample at the time the test sample is placed in the space.

26. A method for monitoring changes in target-analyte concentration within an enclosed space, comprising the steps of:

(a) obtaining an article of commerce according to any one of claims 13-15,

(b) dispensing a known number of probes from the container, constituting a fraction of the total number of commingled probes, into a space to form a sensor comprised of at least one probe,

(c) enclosing the space,

(d) ascertaining target-analyte concentration within the enclosed space over time by:

(i) taking at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation to create an excited sensor, and

(2) measuring radiation emitted by the excited sensor,

(ii) measuring passage of time between at least two of the emission measurements to determine a time interval between identified emission measurement, and

(iii) converting at least the identified emission measurements to a target-analyte concentration based upon a known conversion algorithm, and

(e) reporting at least one of (i) at least two ascertained target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in target-analyte concentration within the enclosed space calculated from data obtained in step (d).

27. A method for measuring concentration of a target-analyte of interest within an enclosed space wherein the target-analyte of interest is selectable from and selectively transitional amongst a plurality of different target-analytes without accessing the space once enclosed, comprising the steps of:

(a) obtaining a supply of target-analyte sensitive probes according to any one of claims 1-12 wherein the supply includes probes that are sensitive to different target-analytes and uniquely interrogatable relative to one another,

(b) placing at least two of the obtained probes sensitive to different target-analytes within a space, to form a sensor sensitive to at least two different target-analytes,

(c) enclosing the space, and

(d) ascertaining concentration of a target-analyte of interest within the enclosed space, selected from the at least two different target-analytes to which the at least two placed probes are sensitive, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the target-analyte of interest,

(ii) measuring radiation emitted by the excited probe sensitive to the target-analyte of interest, and

(iii) converting the measured emission to a concentration of the target-analyte of interest based upon a known conversion algorithm.

28. A method for measuring concentration of at least two different target-analytes within an enclosed space, comprising the steps of:

(a) obtaining a first supply of target-analyte sensitive probes according to any one of claims 1-12 sensitive to a first target-analyte,

(b) obtaining a second supply of target-analyte sensitive probes according to any one of claims 1-12 sensitive to a second target-analyte which is different from the first target-analyte, wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another,

(c) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space, to form a sensor sensitive to both first and second target-analytes,

(d) enclosing the space,

(e) ascertaining concentration of the first target-analyte within the enclosed space, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte,

(ii) measuring radiation emitted by the excited probe sensitive to the first target-analyte, and

(iii) converting the measured emission to a concentration of the first target-analyte based upon a known conversion algorithm, and

(f) ascertaining concentration of the second target-analyte within the enclosed space, by:

(i) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte,

(ii) measuring radiation emitted by the excited probe sensitive to the second target-analyte, and

(iii) converting the measured emission to a concentration of the second target-analyte based upon a known conversion algorithm.

29. A method for monitoring changes in concentration of at least two different target-analytes within an enclosed space, comprising the steps of:

(a) obtaining a first supply of target-analyte sensitive probes according to any one of claims 1-12 sensitive to a first target-analyte,

(b) obtaining a second supply of target-analyte sensitive probes according to any one of claims 1-12 sensitive to a second target-analyte which is different from the first target-analyte, wherein the probes sensitive to a first target-analyte and the probes sensitive to a second target-analyte are uniquely interrogatable relative to one another,

(c) placing at least one probe from the first supply of probes and at least one probe from the second supply of probes within a space to form a sensor sensitive to both first and second target-analytes,

(d) enclosing the space,

(e) ascertaining concentration of the first target-analyte within the enclosed space over time by:

(i) taking a first set of at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the first target-analyte, and

(2) measuring radiation emitted by the excited probe sensitive to the first target-analyte,

(ii) measuring passage of time between at least two of the first set emission measurements to determine a time interval between identified first set emission measurements, and

(iii) converting at least the identified first set emission measurements to a concentration of the first target-analyte based upon a known conversion algorithm,

(f) ascertaining concentration of the second target-analyte within the enclosed space over time by:

(i) taking a second set of at least two emission measurements over time, each measurement comprising the steps of:

(1) exposing the sensor to excitation radiation effective for exciting the probe sensitive to the second target-analyte, and

(2) measuring radiation emitted by the excited probe sensitive to the second target-analyte,

(ii) measuring passage of time between at least two of the second set emission measurements to determine a time interval between identified second set emission measurements, and

(iii) converting at least the identified second set emission measurements to a concentration of the second target-analyte based upon a known conversion algorithm,

(g) reporting at least one of (i) at least two ascertained first target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in first target-analyte concentration within the enclosed space calculated from data obtained in step (e), and

(h) reporting at least one of (i) at least two ascertained second target-analyte concentrations and the time interval between those reported concentrations, and (ii) a rate of change in second target-analyte concentration within the enclosed space calculated from data obtained in step (f).

30. A method of preparing the probe of any of claims 1-12, which includes at least the steps of:

(a) preparing a coating cocktail which contains at least the optically-active target-analyte sensitive material dissolved in a solvent,

(b) applying the cocktail onto the porous scaffold particle, and

(c) allowing the applied cocktail to dry, whereby a solid-state thin film coating of optically-active target-analyte sensitive material is formed on the scaffold particle to form the probe.

31. The method of claim 30 wherein the coating cocktail further includes a polymer operable for forming a target-analyte permeable polymer matrix when dried.

32. The method of claim 30 wherein the scaffold particle is soaked with the cocktail whereby the cocktail penetrates into and coats the pores in the scaffold particle, and the method further includes separating the coated scaffold particle from excess cocktail prior to drying.

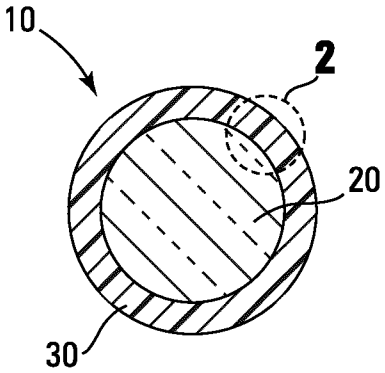


Fig. 1

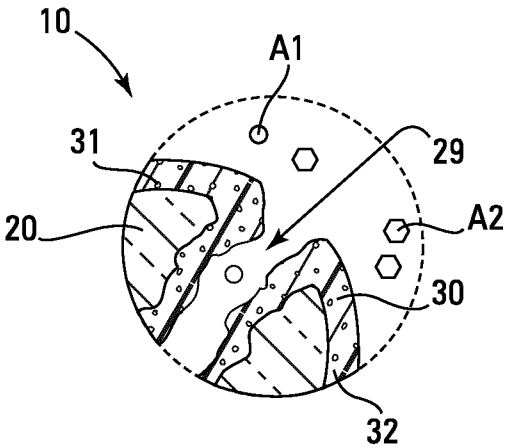


Fig. 2

Fig. 3

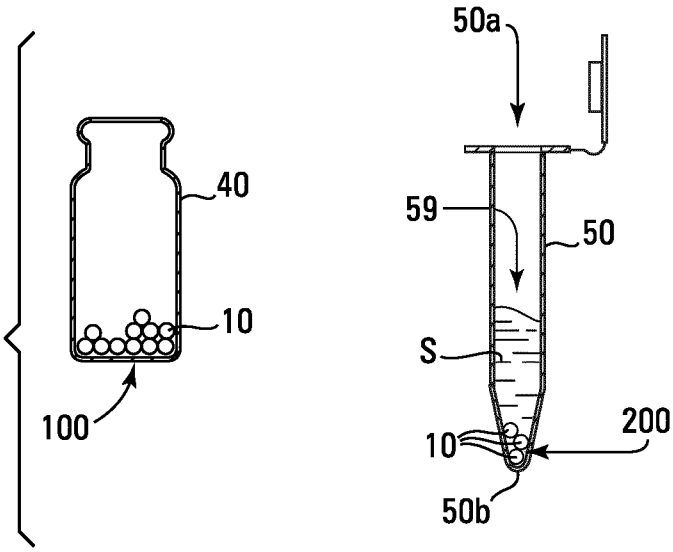


Fig. 4

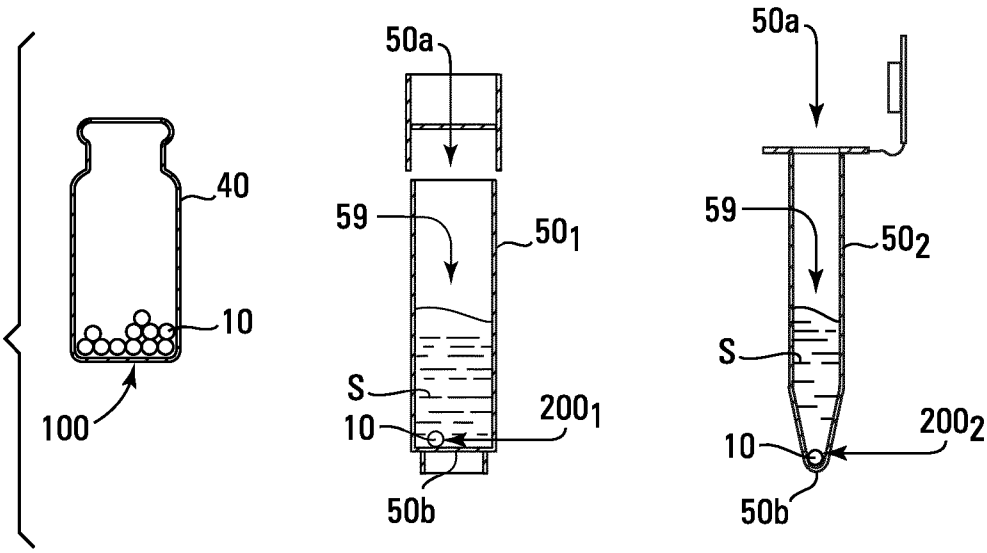
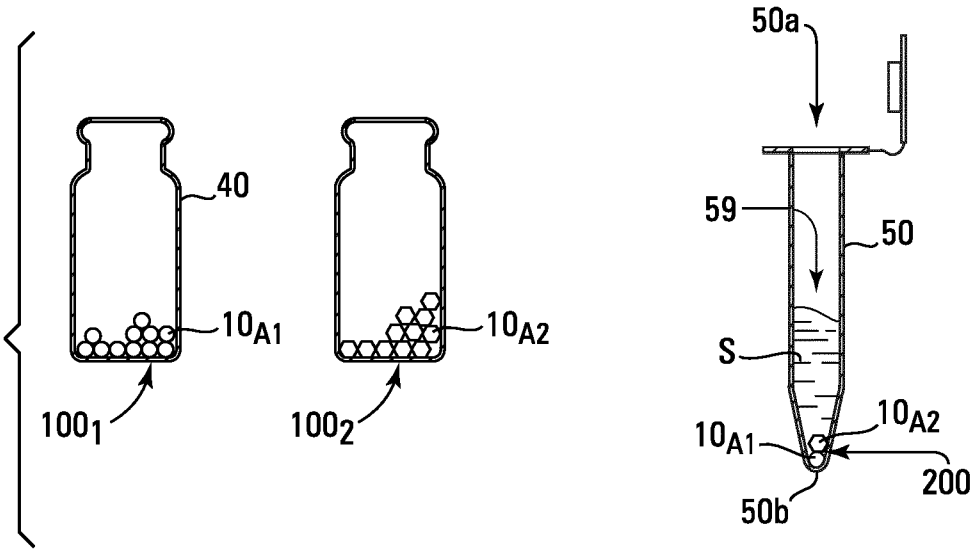


Fig. 5



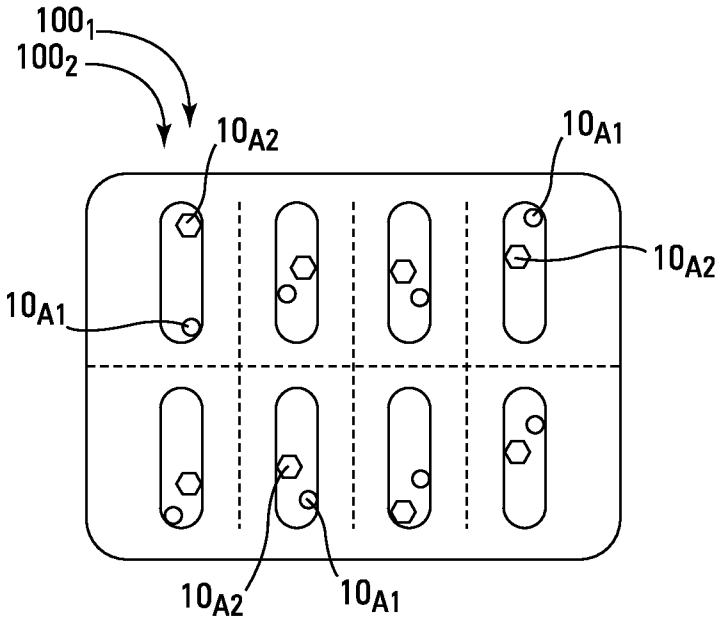


Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/074551

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64 G01N21/77
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C07D B32B

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	paragraph [0018] - paragraph [0021] paragraph [0023] paragraph [0027] paragraph [0031] paragraph [0032] paragraph [0033] -----	17,19,23
Y	US 2009/028756 A1 (SHAHRIARI MAHMOUD R [US]) 29 January 2009 (2009-01-29) the whole document -----	17,19,23
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2013	19/08/2013

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

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/074551

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	PAPKOVSKY D B ET AL: "Biosensors on the basis of luminescent oxygen sensor: the use of microporous light-scattering support materials", SENSORS AND ACTUATORS B: CHEMICAL: INTERNATIONAL JOURNAL DEVOTED TO RESEARCH AND DEVELOPMENT OF PHYSICAL AND CHEMICAL TRANSDUCERS, ELSEVIER S.A, SWITZERLAND, vol. 51, no. 1-3, 31 August 1998 (1998-08-31), pages 137-145, XP004154000, ISSN: 0925-4005, DOI: 10.1016/S0925-4005(98)00171-3 the whole document -----	1

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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