

Analyte sensor and its manufacturing

Description

5 Technical Field

The present invention relates to an analyte sensor comprising a substrate, at least one working electrode, at least one second electrode and a membrane, wherein the membrane is located on top of the at least one second electrode. The present invention further relates to a process for
10 manufacturing the inventive analyte sensor as well as to an analyte sensor system comprising an analyte sensor according to the present invention and an electronics unit. The analyte sensor according to the present invention may mainly be used for conducting an analyte measurement in a body fluid of a user.

15 Background art

Biosensors for measuring analytes in biological fluids, in particular a sensor which is designed for implantation or subcutaneous insertion to measure body fluids, have to fulfill a variety of functions: on the one hand, the sensor must provide for specific and sensitive measurement without interference
20 from e.g. particular components of body fluids. For this purpose, biosensors are frequently covered with membranes excluding particular compounds in order to allow access to the actual sensing sites only for low molecular weight compounds. While the specificity of biosensors is achieved by using of biorecognition elements, such as enzymes, the sensitivity is often tailored by using of diffusion limiting membranes. Finally, the implanted sensor must be biocompatible, thus there is no
25 inflammation reaction of the body caused, for this purpose, an additional biocompatibility membrane may be applied.

Moreover, with implanted sensors, it is preferred to have sensors which can remain in place for a long period without deterioration of the measurement, in order to spare the patient frequently
30 exchanging the sensor.

Implanted sensors, for example, comprise electrode systems which facilitate measurements of physiologically significant analytes such as, for example, like that of glucose in the patient's body. The working electrodes of such a sensor have electrically conductive enzyme layers in which enzyme
35 molecules are bound which release charge carriers by catalytic conversion of the analyte molecules.

In this process, an electrical current is generated as measuring signal whose amplitude correlates to the analyte concentration. These types of sensors are also called electrochemical sensors.

US9895091B2 discloses electrochemical sensors. These electrochemical sensors can comprise an impermeable dielectric layer on top of Ag/AgCl of a reference electrode. This coating is used to extend the reference electrode's lifetime. The electrochemical sensor disclosed has a layered structure wherein the reference electrode is positioned on top of a working electrode. The working electrode is separated from the reference electrode by an insulating layer.

US10470691B2 discloses an analyte sensor which comprises a working electrode and a reference electrode. The sensor may comprise an insulator formed of an insulating material. A portion of the insulator may be removed to expose the working electrode and/or the reference electrode.

The manufacturing of the sensors disclosed in the prior art is very time- and cost-consuming.

Further, they have drawbacks with regard to their long-term stability.

Problem to be solved

It is therefore an object of the present invention to provide an analyte sensor which avoids at least in part certain drawbacks of the prior art, in particular with regard to its manufacturability and also with regard to its long-term stability.

Summary of the invention

This problem is solved by an analyte sensor according to independent claims 1 and 13 as well as by the method for manufacturing this sensor according to independent claim 11 and by the analyte sensor system according to independent claim 14. Preferred embodiments of the invention which may be realized in an isolated way or in any arbitrary combination are disclosed in the dependent claims and throughout the specification

The inventive analyte sensor is particularly easy to manufacture. It furthermore exhibits an excellent long-term stability and a stable sensitivity. In particular, the inventive analyte sensor allows the analyte sensor to comprise only two electrodes instead of three, which makes the inventive analyte sensor particularly cost-efficient. Further, it has an improved biocompatibility

As used in the following, the terms "have", "comprise", or "include" or any arbitrary grammatical variations thereof are used in an exclusive way. Thus, these terms may both refer to a situation in

which, besides the feature introduced by these terms, no further features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions “A has B”, “A comprises B” and “A includes B” may both refer to a situation in which, besides B, no other element is present in A (i. e. a situation in which A solely and
5 exclusively consists of B) and to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C and D or even further elements.

Further, it should be noted that the terms “at least one”, “one or more” or similar expressions indicating that a feature or element may be present once or more than once typically will be used
10 only once when introducing the respective feature or element. In the following, in most cases, when referring to the respective feature or element, the expressions “at least one” or “one or more” will not be repeated, notwithstanding the fact that the respective feature or element may be present once or more than once.

15 Further, as used in the following, the terms “preferably”, “more preferably”, “particularly”, “more particularly”, “specifically”, “more specifically” or similar terms are used in conjunction with optional features, without restricting alternative possibilities. Thus, features introduced by these terms are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by using alternative features.
20 Similarly, features introduced by “in an embodiment of the invention” or similar expressions are intended to be optional features, without any restrictions regarding alternative embodiments of the invention, without any restrictions regarding the scope of the invention and without any restrictions regarding the possibility of combining the features introduced in such way with the optional or nonfunctional features of the invention.

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In a first aspect of the present invention an analyte sensor comprising

- a substrate comprising a first side and a second side;
- at least one working electrode positioned on the first side of the substrate, the at least one working electrode comprising:
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 - at least one electrically conductive material and
 - at least one enzyme;
- at least one second electrode positioned on the second side of the substrate, the at least one second electrode comprising silver;
- a membrane comprising a polymer composition which comprises a hydrophobic polymer,
35 wherein the membrane is located on top of the at least one second electrode is disclosed.

The term "analyte sensor" within the context of the present invention may refer to any device being configured for the detection of an analyte.

5 The term "analyte" may refer to any arbitrary element, component or compound which may be present in a body fluid and the concentration of which may be of interest for the user. Preferably, the analyte may be or may comprise an arbitrary chemical substance or chemical compound which may take part in the metabolism of the user, such as at least one metabolite. As an example, the analyte may be selected from the group consisting of glucose, cholesterol, triglycerides, and lactate. Additionally or alternatively, however, other types of analytes and/or any combination of analytes
10 may be determined. Preferably, the analyte is glucose.

Thus, the analyte sensor is preferably a biosensor. Further preferably, the analyte sensor is an electrochemical sensor. The term "electrochemical sensor" refers to a sensor which is adapted for performing at least one electrochemical measurement, in particular, a plurality or series of
15 electrochemical measurements, in order to detect the analyte comprised within the body fluid by using an amperometric method. Especially, the term "electrochemical measurement" refers to the detection of an electrochemically detectable property of the analyte, such as an electrochemical detection reaction, by employing amperometric methods. Thus, for example, the electrochemical detection may be carried out by applying and comparing one or more electrical potentials.
20 Specifically, the electrochemical sensor may be adapted to generate at least one electrical measurement signal which may directly or indirectly indicate the presence and/or absence of the electrochemical detection reaction, such as at least one current signal and/or at least one voltage signal. The measurement may be a quantitative and/or a qualitative measurement.

25 In a particularly preferred embodiment of the present invention, the analyte sensor may be fully or partially implantable and may, thus, be adapted for performing the detection of the analyte in the body fluid in the subcutaneous tissue, in particular, an interstitial fluid. As used herein the terms "implantable" or "subcutaneous" refer to be fully or at least partially arranged within the body tissue of the user, preferably partially arranged within the body tissue of the user. For this purpose,
30 the analyte sensor may comprise an insertable portion, wherein the term "insertable portion" may generally refer to a part or component of an element configured to be insertable into an arbitrary body tissue while other parts or components may remain outside of the body tissue. Preferably, the insertable portion may fully or partially comprise a biocompatible membrane, i. e. a surface which may have as little detrimental effects on the user, the patient, or the body tissue as possible, at least
35 during typical durations of use.

Thus, preferably, the analyte sensor of the present invention is an implantable sensor.

As generally used, the term "body fluid" may refer to fluid, in particular liquid, which may typically be present in a body or a body tissue of the user or the patient and/or which may be produced by the body of the user or the patient. Preferably, the body fluid may be selected from the group consisting of blood and interstitial fluid. However, additionally or alternatively, one or more other types of body fluid may be used, such as saliva, tear fluid, urine or other body fluids. During the detection of the analyte, the body fluid may be present within the body or body tissue. Thus, the analyte sensor may be configured for detecting the analyte within the body tissue. The analyte sensor is in one embodiment suitable for short-term application, e. g. 3 to 21 days, or for long-term application e. g. 1 to 12 months. During its application, the analyte may be determined by continuous or discontinuous measurements.

The analyte sensor of the invention is an electrochemical sensor comprising at least one working electrode and at least one second electrode. More particularly, the sensor is an amperometric electrochemical sensor comprising the at least one working electrode and the at least one second electrode. The working electrode is sensitive for the analyte to be measured at a polarization voltage which may be applied between the at least one working electrode and the at least one second electrode and which may be regulated by a potentiostat. A measurement signal may be provided as an electric current between the at least one working electrode and the at least one second electrode.

The inventive analyte sensor comprises a substrate which comprises a first side and a second side.

Within the context of the present invention, the term "substrate" specifically may refer, without limitation, to any kind of material or combination of materials which is suitable to form a carrier layer to support the at least one working electrode and the at least one second electrode. In particular the substrate may comprise an electrically insulating material. Within the context of the present invention "electrically insulating material" is a broad term and given its ordinary and customary meaning to a person of ordinary skill in the art. The term "electrically insulating material" may also encompass a dielectric material. The term specifically may refer, without limitation, to a material or combination of materials which prevent the transfer of electrical charges and which do not sustain a significant electrical current. Specifically, without limiting other possibilities, the at least one electrically insulating material may be or may comprise at least one insulating resin such as insulating epoxy resins used in manufacturing of electronic printed circuit boards. In particular, it may comprise or be at least one thermoplastic material such as a polycarbonate, a polyester, a polyvinylchloride, a polyurethane, a polyethylene, a polypropylene, polystyrene, a polyether, a polyamide, a polyimide, polytetrafluoroethylene or a copolymer thereof.

In an embodiment, the at least one electrically insulating material may comprise or may be alumina. Suitable polyesters are, for example, selected from the group consisting of polyethylene terephthalate (PET), glycol modified polyethylene terephthalate, and polyethylene naphthalate. A suitable polyethylene is for example selected from the group consisting of high density
5 polyethylene (HDPE) and low density polyethylene (LDPE).

Thus in a preferred embodiment the substrate comprises at least one electrically insulating material selected from the group consisting of an insulating epoxy resin, a polycarbonate, a polyester, a polyvinylchloride, a polyurethane, a polyethylene, a polypropylene, polystyrene, a polyether, a
10 polyamide, a polyimide, polytetrafluoroethylene or a copolymer thereof, and alumina.

The substrate comprises a first side and a second side. To the person skilled in the art it is clear that the first side and the second side are different from one another.

15 In an embodiment the first side and the second side are positioned opposite each other. Therefore, in an embodiment the substrate comprises two opposing sides, the first side and the second side opposing the first side.

Thus it is preferred that in the analyte sensor according to the present invention the first side and
20 the second side of the substrate are positioned opposite each other.

The substrate may be flat substrate. Specifically the substrate may be flexible and/or deformable. In particular, the substrate may be bendable. Thus, as an example, the substrate may be a thin, flexible substrate. As an example, the substrate may have a thickness of 50 μm to 1 mm, specifically a
25 thickness of 80 μm to 500 μm , such as 110 μm to 250 μm .

The substrate may have a length which is preferably less than 50 mm, such as a length of 30 mm or less, e.g. a length of 5 mm to 30 mm.

30 If the analyte sensor is an implantable sensor, preferably a partially implantable sensor, then the length of the substrate is measured in the insertion direction of the analyte sensor. The length of the substrate refers to the total length of the substrate. The “total length of the substrate” is the overall length of the substrate, including the insertable portion of the substrate which is within the body tissue of the user during use of the analyte sensor and the on body portion of the substrate. The “on
35 body portion of the substrate” is the portion of the substrate which may, for example be connected to the electronics unit.

The analyte sensor comprises at least one working electrode positioned on the first side of the substrate. Preferably the at least one working electrode is positioned only on the first side of the substrate. This means, within the context of the present invention, that in an embodiment the second side does not comprise at least one working electrode.

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The at least one working electrode is preferably adapted for detecting the analyte, in particular, the at least one working electrode is an electrode of the analyte sensor which is sensitive for the analyte.

10 The at least one working electrode comprises at least one electrically conductive material. "Electrically conductive material" within the context of the present invention refers to a material being capable of sustaining an electrical current. Thus, the at least one electrically conductive material may be selected from the group consisting of metals, and nonmetallic electrically conductive materials.

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Suitable metals are known as such and are, for example, selected from the group consisting of gold, nickel, platinum, and palladium, wherein gold is particularly preferred.

20 Suitable nonmetallic electrically conductive materials are for example selected from the group consisting of carbon, carbon paste, gold paste or conductive polymers. Suitable conductive polymers are, for example polyaniline and/or poly-3,4-ethylenedioxythiophene (PEDOT). Carbon paste may comprise, for example, carbon and a solvent such as diethylene glycol butyl ether and at least one binder such as vinyl chloride co- and terpolymers. Carbon paste is known as such.

25 Thus, the at least one electrically conductive material of the at least one working electrode preferably is selected from the group consisting of gold, nickel, platinum, palladium, carbon, carbon paste, polyaniline and poly-3,4-ethylenedioxythiophene (PEDOT), particularly preferred, the at least one electrically conductive material of the at least one working electrode is selected from the group consisting of gold, carbon, and carbon paste. More preferably, the at least one
30 electrically conductive material consists essentially of gold and/or carbon and/or carbon paste. In an embodiment, the at least one electrically conductive material has a layered structure wherein a first layer consists of gold and a second layer consists of carbon and/or carbon paste. In this embodiment, preferably, gold is positioned on top of the first side of the substrate and on top of the gold, carbon and/or carbon paste is positioned.

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In particular, the at least one working electrode may comprise the at least one electrically conductive material in the form of at least one conductive trace. The term "conductive trace" within

the context of the present invention refers, without limitations, to an electrically conductive strip, layer, wire or other type of electrical conductor. The conductive trace may have a thickness of at least 0.05 μm , preferably of at least 0.5 μm , more preferably of at least 5 μm , specifically of at least 7 μm , or at least 10 μm . In the case where the conductive trace comprises carbon or is carbon, the

5 conductive trace may specifically have a thickness of at least 7 μm , more specifically of at least 10 μm . Specifically, in the case where the conductive trace is gold, the conductive trace may have a thickness of at least 50 nm, more specifically of at least 900 nm.

The at least one electrically conductive material may be positioned on the first side of the substrate

10 by any known method, for example via chemical vapor deposition (CVD), physical vapor deposition (PVD), or a wet-coating process. Wet-coating processes are known as such. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing.

15 The at least one working electrode comprises at least one enzyme. The at least one working electrode may comprise precisely one enzyme or a mixture of two or more enzymes. Precisely one enzyme is preferred. Specifically, the enzyme is capable of catalyzing a chemical reaction converting the analyte. Even more specifically the at least one enzyme is selected from the group consisting of a glucose oxidase (EC 1.1.3.4), a hexose oxidase (EC 1.1.3.5), an (S)-2 hydroxy acid

20 oxidase (EC 1.1.3.15), a cholesterol oxidase (EC 1.1.3.6), a glucose dehydrogenase, a galactose oxidase (EC 1.1.3.9), an alcohol oxidase (EC 1.1.3.13), an L-glutamate oxidase (EC 1.4.3.11), and an L-aspartate oxidase (EC 1.4.3.16). In particular, the at least one enzyme is a glucose oxidase (GOx) and/or modifications thereof.

25 The at least one enzyme may be comprised in a sensing material. The sensing material which comprises the at least one enzyme may be located at least partially on the electrically conductive material of the at least one working electrode. In particular, the sensing material may cover at least a portion of the at least one conductive trace. The sensing material in conjunction with the conductive trace forms the at least one working electrode. In particular, the sensing material

30 preferably forms a layer on the at least one electrically conductive material.

The sensing material may be applied by any known method to the at least one electrically conductive material, for example by a wet-coating process. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing,

35 dispensing, slot-coating, dip coating and screen printing. After the wet-coating process, the layer of the sensing material may be further treated. Such treatments are for example drying treatment, curing treatments and/or laser ablation treatments. Such treatments are known as such.

The term “sensing material”, as used herein, is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a material that may be
5 or may comprise at least a polymeric material; specifically it may be or may comprise at least a polymeric material and at least a metal containing complex. The metal containing complex may be selected from the group of transition metal element complexes, specifically the metal containing complex may be selected from osmium-complexes, ruthenium-complexes, vanadium-complexes, cobalt-complexes, and iron-complexes, such as ferrocenes, such as 2-aminoethylferrocene. Even
10 more specifically, the sensing material may be a polymeric transition metal complex as described for example in WO 01/36660 A2, the content of which is included by reference. In particular, the sensing material may comprise a modified poly(vinylpyridine) backbone loaded with poly(bi-imidizyl) Os complexes covalently coupled through a bidentate linkage. A suitable sensing material is further described in Feldmann et al, *Diabetes Technology & Therapeutics*, 5 (5), 2003, 769-779,
15 the content of which is included by reference. Suitable sensing materials further may include ferrocene-containing polyacrylamide-based viologen-modified redox polymer, pyrrole-2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)(ABTS)-pyrene, Naphthoquinone-LPEI. The polymeric transition metal complex may represent a redox mediator incorporated into a cross-linked redox polymer network. This is advantageous as it may facilitate electron transfer between the at least one
20 enzyme or analyte and the conductive trace. In order to avoid a sensor drift, the redox mediator and the enzyme may be covalently incorporated into a polymeric structure.

In an embodiment the sensing material may comprise a polymeric material and MnO₂-particles or any other material catalyzing hydrogen peroxide oxidation reaction as well as the at least one
25 enzyme. Another material catalyzing hydrogen peroxide oxidation reaction is Pt (platinum).

Moreover, the sensing material may additionally comprise at least one crosslinker; the crosslinker may for example be capable of crosslinking at least part of the sensing material. Specifically the sensing material may comprise at least one crosslinker selected from UV-curable crosslinkers and
30 chemical crosslinkers; more specifically the sensing material comprises a chemical crosslinker. Alternatively, the sensing material may be free of any crosslinker. “Free of any crosslinker” as used herein, is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer to a concentration of crosslinker in the range from 0 to 0.5 wt-% based on
35 the dry weight of the sensing material. The term “dry weight” as used herein refers to the dry matter of the respective material, e.g. the material without the addition of any water or other solvent.

Suitable chemical crosslinkers according to the present invention are preferably selected from the group consisting of epoxide based crosslinkers, such as diglycidyl ethers like poly(ethylene glycol) di-glycidyl ether (PEG-DGE) and poly(propylene glycol) diglycidyl ether; trifunctional short chain
5 epoxides; anhydrides; diglycidyl ethers such as Resorcinol diglycidyl ether, Bisphenol A diglycidyl ether, Diglycidyl 1,2-cyclohexanedicarboxylate, Poly(ethylene glycol) diglycidyl ether, Glycerol diglycidyl ether, 1,4-Butanediol diglycidyl ether, Poly(propylene glycol) diglycidyl ether, Bisphenol diglycidyl ether, Poly(dimethylsiloxane), diglycidyl ether, Neopentyl glycol diglycidyl ether, 1,2,7,8-Diepoxyoctane, 1,3-Glycidioxypropyl-1,1,3,3-Tetramethyldisioxane; triglycidyl
10 ethers such as N,N-Diglycidyl-4-glycidyl-o-aniline, Trimethylolpropane triglycidyl ether; and tetraglycidyl ethers such as Tetrakisepoxy cyclosiloxane, Pentaerythritol tetraglycidyl ether, tetraglycidyl-4,4'-methylenebisbenzamine.

The term “chemical crosslinker” as used herein, is a broad term and is to be given its ordinary and
15 customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a crosslinker that is capable of initiating a chemical reaction generating a crosslinked molecular network and/ or a cross-linked polymer when exposed to heat. “Exposed to heat” may refer to being exposed to a temperature above 15°C, specifically to a temperature above 20 °C; more specifically to a
20 temperature in the range from 20 °C to 50 °C and even more specifically to a temperature in the range from 20 °C to 25 °C. More specifically, chemical crosslinkers may initiate crosslinking of the sensing material when exposed to heat.

The term “UV-curable crosslinker” as used herein, is a broad term and is to be given its ordinary
25 and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to the ability of a chemical substance of initiating a photochemical reaction generating a crosslinked molecular network and/ or a crosslinked polymer when irradiated by light in the UV spectral range. More specifically, UV-curable crosslinkers may initiate crosslinking of the layer of the sensing material
30 when irradiated by UV light.

Suitable UV curable crosslinkers according to the present invention include: benzophenone, diazirine and azide. Particularly suitable UV-curable crosslinkers are for example selected from the group consisting of, benzophenone comprising cross-linkers, poly(Di(2-hydroxy 3
35 aminobenzophenonepropylene) glycol), Dibenzophenone 1,2-cyclohexanedicarboxylate, Bis[2-(4-azidosalicylamido)ethyl] Disulfide, reaction products of the reaction of 4-aminobenzophenone with any one of the above for the chemical cross-linker described di-glycidyl cross-linkers, triglycidyl

cross-linkers and tetraglycidyl cross-linkers, an example of such a reaction product is 2,4,6,8-Tetramethyl-2,4,6,8-tetrakis(2-hydroxy 3-aminopropylbenzophenone)-cyclotetrasiloxan, and reaction products of the reaction of 4-Benzoylbenzoic Acid N-Succinimidyl Ester with a diamine or a Jeffamine.

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The analyte sensor furthermore comprises at least one second electrode positioned on the second side of the substrate. Preferably, the at least one second electrode is positioned only on the second side of the substrate. This means, within the context of the present invention, that in an embodiment, the first side does not comprise at least one second electrode. In an embodiment,
10 precisely one second electrode is positioned on the second side of the substrate. Preferably, the at least one second electrode does not comprise an enzyme. Thus, preferably, the at least one second electrode is free of the at least one enzyme. Preferably, the second side of the substrate is free of enzyme.

15 The at least one second electrode may be selected from the group consisting of a counter electrode, a reference electrode, and a combined counter/reference electrode. Preferably, the at least one second electrode is a combined counter/reference electrode.

Thus, it is preferred that the at least one second electrode of the analyte sensor is selected from the
20 group consisting of a counter electrode, a reference electrode and a combined counter/reference electrode.

The at least one second electrode comprises silver. "Silver" within the context of the present invention does not only encompass elemental silver, but also any silver containing compound.
25 Therefore, the at least one second electrode comprises elemental silver and/or at least one silver containing compound. A preferred silver containing compound is silver chloride (AgCl). For example, the at least one second electrode comprises elemental silver and/or silver chloride. In particular, the at least one second electrode comprises elemental silver and silver chloride. In particular, the at least one second electrode comprises silver / silver chloride (Ag/AgCl). In an
30 embodiment, the at least one second electrode only comprises AgCl when the analyte sensor is manufactured. No elemental Ag is added when the analyte sensor is manufactured. During use of the analyte sensor, elemental Ag may then be formed from AgCl, so that during use, the analyte sensor comprises Ag/AgCl. The reaction of AgCl to form elemental Ag is known to the skilled person as such.

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Thus, an analyte sensor which comprises at least one second electrode which comprises Ag/AgCl is preferred.

For example, the load of AgCl of the at least one second electrode is in the range from 20 µg to 150 µg. If two or more second electrodes are comprised, then the load of AgCl of the at least one second electrode refers to the sum of the load of AgCl of the two or more second electrodes. The load of AgCl of the at least one second electrode refers to the load when the analyte sensor is manufactured. It is clear to the skilled person that the load may change during use of the analyte sensor, for example due to the formation of elemental Ag from AgCl.

Thus, an analyte sensor in which the load of AgCl of the at least one second electrode is in the range from 20 µg to 150 µg is preferred.

The minimum load of AgCl of the at least one second electrode may be calculated according to the following formula.

$$m(\text{AgCl}) = \frac{M(\text{AgCl}) * I * t}{z * F}$$

wherein

I is the highest possible current in A while the analyte sensor is in use

t is the total wear time of the sensor in s

F is the Faraday constant in C/mol

z is the charge number of silver (z = 1)

M(AgCl) is the molar mass of AgCl

m(AgCl) is the load of AgCl of the at least one second electrode.

Ag/AgCl which the at least one second electrode in an embodiment comprises may be comprised in a binder. Suitable binders are known as such and are, for example, selected from the group consisting of metallic binders, ceramic binders and polymeric binders. Preferred are polymeric binders, in particular physically binding polymer binders and/or chemically binding polymer binders.

For example, Ag/AgCl which the at least one second electrode comprises, comprises in the range from 50 to 70 wt.-% of Ag, in the range from 20 to 40 wt.-% of AgCl and in the range of 1 to 20 wt.-% of a binder, wherein the wt.-% are in each case based on the sum of the wt.-% of Ag, AgCl and the binder.

The at least one second electrode may comprise at least one second conductive trace. The at least one second conductive trace is preferably positioned on the second side of the substrate. In particular, the first side of the substrate preferably does not comprise a second conductive trace.

The term "second conductive trace" specifically may refer, without limitation, to an electrically conductive strip, layer, wire or other type of elongated electrical conductor. In particular, the term may refer to at least one second electrically conductive material. Hence, the at least one second

5 conductive trace is preferably capable of sustaining an electrical current. For example, the at least one second electrically conductive material may be selected from the group consisting of gold, nickel, platinum, palladium, carbon, carbon paste, polyaniline and poly-3,4-ethylenedioxythiophene (PEDOT). Particularly preferred, the at least one second electrically

10 conductive material of the at least one second electrode is selected from the group consisting of gold, carbon, and carbon paste. More preferably, the at least one second electrically conductive material consists essentially of gold and/or carbon and/or carbon paste. In an embodiment, the at least one second electrically conductive material has a layered structure wherein a first layer consists of gold and a second layer consists of carbon and/or carbon paste. In this embodiment, preferably, gold is positioned on top of the second side of the substrate and on top of the gold,

15 carbon and/or carbon paste is positioned.

It is preferred that silver which the at least one second electrode comprises is positioned on top of the at least one second conductive trace. Thus, it is preferred, that Ag/AgCl is positioned at least partially on top of the at least one second conductive trace, in particular on top of the layered

20 structure of the at least one second electrically conductive material. Silver, preferably Ag/AgCl, and the at least one second conductive trace, in particular the layered structure of the at least one second conductive material, form the at least one second electrode.

Thus, the silver which the at least one second electrode comprises typically forms a layer. The

25 thickness of the layer is for example in the range from 5 μm to 30 μm .

The at least one second conductive trace and the at least one second electrically conductive material may be applied to the second side of the substrate by the same methods as the methods by which the at least one conductive trace and the at least one electrically conductive material of the at

30 least one working electrode are applied to the first side of the substrate. Thus, the embodiments and preferences described above hold true. The methods for applying the at least one second conductive trace and the at least one second electrically conductive material of the second electrode and the methods for applying the at least one conductive trace and the at least one electrically conductive material of the working electrode may be selected independent from one another.

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Silver, in particular Ag/AgCl, comprised in the at least one second electrode may be applied to the second side of the substrate, in particular at least partially on top of the at least one second

conductive trace, by the same methods as the methods by which the sensing material of the at least one working electrode is applied to the first side of the substrate. Thus, the embodiments and preferences described above hold true. The method for applying silver, in particular Ag/AgCl, comprised in the at least one second electrode and the method for applying the sensing material
5 comprised preferably in the at least one working electrode may be selected independent from one another.

The analyte sensor of the present invention comprises a membrane. The membrane comprises a polymer composition which comprises a hydrophobic polymer and is located on top of the at least
10 one second electrode.

In an embodiment, the term “membrane” within the context of the present invention refers to a layer of at least one material which provides a selective barrier. Thus, such a membrane generally may selectively allow for one or more molecules and/or compounds to pass through the membrane,
15 whereas other molecules and/or compounds are stopped by the membrane. For example, the membrane allows transfer of water and/or chloride anions through the membrane and restricts transfer of silver cations and/or silver chloride through the membrane.

For example, in this embodiment, the membrane may have a thickness in the range from 1 μm to 100 μm , preferably in the range from 5 μm to 20 μm .
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In another embodiment, which is particularly preferred, the term “membrane” within the context of the present invention refers to a layer of at least one material which is essentially impermeable. “Essentially impermeable” means that the membrane has a water uptake of less than 1 % by
25 weight, based on the total weight of the membrane.

For example, the membrane may have a thickness in the range from 1 μm to 100 μm , preferably in the range from 5 μm to 15 μm .

30 The embodiments described in the following with regard to the membrane apply to both embodiments of the membrane, preferably to embodiment of the membrane referring to a layer of at least one material which is essentially impermeable.

The membrane is located on top of the at least one second electrode.

35 “Located on top of the at least one second electrode” means that the membrane covers the at least one second electrode. In an embodiment, the membrane covers the at least one second electrode

fully. The term “cover fully” within the context of the present invention means that the at least one second electrode, when the analyte sensor is implanted into the user, does not have direct contact with the body fluid of the patient. Instead, only the membrane has direct contact with the body fluid.

5

In a further embodiment, the membrane covers the at least one second electrode partially. The term “covers partially” within the context of the present invention means that the at least one second electrode, when the analyte sensor is implanted into the user, does have direct contact with the body fluid. In particular, the at least one second electrode has direct contact with the body fluid via
10 holes in the membrane. This embodiment is particularly preferred if the term “membrane” refers to a layer of at least one material which is essentially impermeable.

Thus in an embodiment, the membrane comprises holes. The membrane may comprise precisely one hole or two or more holes.

15

The term “holes” within the context of the present invention is given its ordinary and customary meaning to a person skilled in the art. In particular, it means any opening and/or perforation within the membrane. The holes allow passage of one or more molecules and/or compounds through the holes. Thus, the holes provide a fluidic channel between the at least one second electrode and the
20 body fluid.

The holes may have any shape and any size. The holes may be positioned anywhere within the membrane. For example, the holes may be positioned at an edge of the membrane and/or essentially in the center of the membrane.

25

For example, the membrane comprises holes, wherein the total area of the holes comprised in the membrane is at most 0.15 square millimeters (mm²), preferably at most 0.05 mm². In an embodiment, the total area of holes comprised in the membrane is in the range from 0.005 to 0.15 mm², preferably in the range from 0.005 to 0.05 mm².

30

Thus, an analyte sensor is preferred in which the membrane comprises holes, wherein the total area of holes has a size of at most 0.15 mm².

Within the context on the present invention, “the total area of holes” relates to the sum of the
35 surface area of the holes.

In a preferred embodiment of the present invention, the at least one working electrode does not comprise the membrane comprising the polymer composition which comprises the hydrophobic polymer.

- 5 Thus, an analyte sensor is preferred in which the at least one working electrode is free of the membrane comprising the polymer composition which comprises the hydrophobic polymer.

The membrane comprises a polymer composition which comprises a hydrophobic polymer.

- 10 “Hydrophobic” within the context of the present invention means that the polymer has a water uptake in the range from 0 to 5 % by weight, in an embodiment a water uptake of less than 1 % by weight, based on the total weight of the polymer.

- Thus, an analyte sensor is preferred, wherein the hydrophobic polymer has a water uptake of less
15 than 1 % by weight, based on the total weight of the hydrophobic polymer.

The hydrophobic polymer is preferably a thermoplastic hydrophobic polymer.

- The hydrophobic polymer, for example, has a glass transition temperature in the range from -
20 100 °C to 0 °C, preferably in the range from -70 °C to -50 °C. The glass transition temperature may be measured via differential scanning calorimetry using a ramp of 10 °C/min for heating and cooling. The glass transition temperature is measured during the second heating cycle. This means that first, the hydrophobic polymer is heated with a ramp of 10 °C/min, then it is cooled with a ramp of 10 °C/min and then it is heated again with a ramp of 10 °C/min to determine the glass
25 transition temperature.

- The hydrophobic polymer, for example, has a crystallization temperature in the range from 50 °C to 100 °C, for example in the range from 75 °C to 85 °C. The crystallization temperature is measured via differential scanning calorimetry using the same parameters as for the glass transition
30 temperature.

Thus, an analyte sensor is preferred wherein the hydrophobic polymer has a glass transition temperature, wherein the glass transition temperature is in the range from -100 °C to 0 °C.

- 35 This glass transition temperature is particularly advantageous as it results in a sufficiently high stability of the membrane. If the sensor is bent, in particular during use, the membrane will not or only to a small extend be damaged.

The hydrophobic polymer may be any hydrophobic polymer known to the skilled person.

Preferably, the hydrophobic polymer is selected from the group consisting of thermoplastic polyurethanes (TPU), thermoplastic polyurea, polyethylene, polypropylene, polystyrene, butyl methacrylate polymers (BUMA), polyethylene terephthalate (PET), and UV hardening resins, such as acrylated silicones, acrylated urethanes, acrylated polyesters and/or acrylated epoxides.

Preferably, the hydrophobic polymer is a thermoplastic polyurethane.

Therefore, an analyte sensor is preferred, wherein the polymer composition comprises a

hydrophobic thermoplastic polyurethane.

The hydrophobic thermoplastic polyurethane may comprise hard segments and soft segments in various ratios. Suitable hard segments usually comprise a polymerization product of a diisocyanate and a polyol. A suitable diisocyanate may be an aliphatic diisocyanate or an aromatic diisocyanate, preferably an aliphatic diisocyanate.

Suitable aromatic diisocyanates are for example, 4,4'-methylene diphenyl diisocyanate, and/or toluene-2,4-diisocyanate.

Suitable aliphatic diisocyanates are for example, hexamethylene diisocyanate, and/or isophorone diisocyanate.

A suitable polyol is preferably a diol, such as 1,4-butanediol, 1,5-pentanediol, 1,6-hexanediol, and/or 1,10-decanediol.

Suitable soft segments may comprise polyethers and/or polyesters. Suitable polyethers are for example polyethylene oxide and/or polytetrahydrofuran, whereas suitable polyesters are for example polyethylene terephthalate and/or polyethylene naphthalate.

The polymer composition may comprise further components.

The analyte sensor may comprise at least one third electrode. Preferably, the analyte sensor does not comprise at least one third electrode.

If at least one third electrode is comprised in the analyte sensor, then the at least one second electrode is preferably selected from the group consisting of a counter electrode and a reference electrode. The at least one third electrode is then preferably also selected from the group consisting

of a counter electrode and a reference electrode. If the at least one second electrode is a counter electrode, then the at least one third electrode is a reference electrode and vice versa.

The analyte sensor may further comprise at least one flux limiting membrane.

5

The at least one flux limiting membrane is specifically at least positioned on top of the at least one working electrode. Preferably, the at least one flux limiting membrane is also positioned on top of the membrane comprising the polymer composition which comprises the hydrophobic polymer.

10 The term “flux limiting membrane”, as used herein, is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a layer of at least one material, which provides a selective barrier. Thus, the flux limiting membrane generally may selectively allow for one or more molecules and/or compounds to pass, whereas other molecules
15 and/or compounds are stopped by the flux limiting membrane. Thus, the flux limiting membrane is permeable for the at least one analyte to be detected. Thus, as an example, the flux limiting membrane may be permeable for one or more of glucose, lactate, cholesterol or other types of analytes. The at least one flux limiting membrane may, hence, function as a diffusion barrier that controls diffusion of the analyte from the exterior, e.g. the body fluid surrounding the analyte
20 sensor, to the sensing material, i. e. the at least one enzyme which the at least one working electrode comprises. In addition, the at least one flux limiting membrane may function as a biocompatibility membrane layer as mentioned elsewhere herein.

The at least one flux limiting membrane, as an example, may have a thickness sufficient for
25 providing mechanical stability. The at least one flux limiting membrane specifically may have a thickness of 1 μm to 150 μm . For the at least one flux limiting membrane, as outlined herein, several materials may be used, standalone or in combination. Thus, as an example, the flux limiting membrane specifically may comprise at least one polymeric material. Suitable polymeric materials may, for example be selected from the group consisting of a polyvinylpyridine based copolymer, a
30 polyurethane and a hydrogel. Polyvinylpyridine based copolymers are particularly suitable.

Suitable hydrogels are in particular polyethylene glycol copolymers (PEG copolymers), polyvinyl acetate copolymers (PVA copolymers), poly(2-alkyl-2-oxazoline) copolymers, polyacrylate and/or methacrylate-acrylate copolymers or block-copolymers, in particular polyacrylate and/or
35 methacrylate-acrylate copolymers or block-copolymers comprising hydrophilic side groups. Thus, as an example, suitable hydrogels may be selected from the group consisting of (hydroxyethyl)methacrylate (HEMA) -homopolymers, HEMA-copolymers, silicon hydrogels and

HEMA-co-N-vinylpyrrolidone-polymers, each of which may comprise side groups selected from the group consisting of methacrylic acid, glycerol methacrylate, N,N-dimethylacrylamide and phosphorylcholine.

- 5 These types of flux limiting membranes are generally known in the art. As an example, flux limiting membranes as disclosed in e.g. EP2697388 A1, WO 2007/071562 A1 and/or in WO 2005/078424 A1 may be used. Specifically, the polymeric material may have a weight average molecular weight (MW) of more than 10.000 kDa. More specifically, the polymeric material may have a weight average molecular weight (MW) of more than 50.000 kDa, or even more than
- 10 100.000 kDa. Particularly suitable are polymeric materials with a weight average molecular weight (MW) of 10.000 to 500.000 kDa. The polymeric material of the flux limiting membrane may be the same as or may be different from the polymeric material of the sensing material.

The analyte sensor may further comprise at least one biocompatibility membrane.

- 15 The at least one biocompatibility membrane is specifically at least positioned on top of the at least one working electrode. Preferably, the at least one biocompatibility membrane is also positioned on top of the membrane comprising the polymer composition which comprises the hydrophobic polymer. In particular, the at least one biocompatibility membrane is positioned on top of the flux
- 20 limiting membrane which is in an embodiment of the present invention comprised in the analyte sensor. Specifically, the biocompatibility membrane fully covers the at least one flux limiting membrane. The term "fully covers" within the context of the present invention means that, specifically, the flux limiting membrane is not in direct contact with a body fluid if the analyte sensor is in use, but only the biocompatibility membrane is in direct contact with the body fluid.
- 25 This means that at least the implantable portion of the analyte sensor is preferably fully covered by the at least one biocompatibility membrane.

- The term "biocompatibility membrane ", also denoted biocompatibility layer, as used herein, relates to a layer, in particular an outmost layer of the analyte sensor or part thereof, consisting of a
- 30 biocompatible material. Specifically, the biocompatibility layer has a thickness of from 1 μm to 10 μm , in an embodiment of from 3 μm to 6 μm . More specifically, the biocompatibility layer covers the analyte sensor at least partly or completely. Even more specifically, the biocompatibility layer may be the outmost layer of the analyte sensor. Thus, even more specifically, at least a part of the biocompatibility layer contacts a body fluid of a subject. For example, the biocompatibility layer
- 35 may be not diffusion-limiting for the analyte as specified elsewhere herein. For example, the biocompatibility layer may be not diffusion-limiting for small molecules having a molecular weight of less than 2.000 Da, in an embodiment less than 1.000 Da. For example, the biocompatibility

layer may not comprise an added enzyme. For example, the biocompatibility layer may not comprise an added polypeptide. As will be understood by the skilled person, this does not exclude that enzyme or polypeptide molecules diffuse into the biocompatibility layer from adjacent layers, tissues, or body fluids.

5

The term “biocompatible material”, as used herein relates to a material suitable for use with living tissue or a living system by not being or being to a reduced extent toxic, injurious, or physiologically reactive and/or causing to a reduced extent or not causing immunological rejection. In an embodiment, the biocompatible material is a material not inducing a bodily response, e.g. an inert material or a material comprising chemical compounds preventing bodily responses from occurring in the vicinity of the biocompatibility layer. In another embodiment, the biocompatible material is a material pre-venting cells from attaching to said biocompatibility layer. The biocompatibility membrane may be or may comprise at least one material selected from the group consisting of methacrylate based polymers and copolymers, such as acrylamide-methacrylate based copolymers, biodegradable polysaccharides such as hyaluronic acid (HA), agarose, dextran and chitosan. Further biocompatible materials are disclosed in WO 2019/166394 A1 and include nonbiodegradable synthetic hydrogels such as hydrogels prepared from the copolymerization of 2-hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), acrylamide (AAm), acrylic acid (AAc), N-isopropylacrylamide (NIPAm), and methoxyl poly(ethylene glycol) (PEG) monoacrylate (mPEGMA or PEGMA), with cross-linkers, such as N,N'-methylenebis(acrylamide) (MBA), ethylene glycol diacrylate (EGDA) and PEG diacrylate (PEGDA), Pluronic® polymers with a structure of poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO)-PEO, chemical cross-linking of modified poly(vinyl alcohol) (PVA), Poly (4vinylpyridine), PEG.

- 25 Another object of the present invention is a method for manufacturing an analyte sensor, in particular the inventive analyte sensor, the method comprising the steps:
- a) providing a raw substrate which comprises a first side and a second side,
 - b) preparing a working electrode region on the first side of the raw substrate, the preparing of the working electrode region comprising the steps:
 - 30 b1) applying an electrically conductive material to the first side of the raw substrate,
 - b2) applying a sensing material comprising at least one enzyme at least partially on the electrically conductive material,
 - c) preparing a second electrode region on the second side of the raw substrate, the preparing of the second electrode region comprising the steps:
 - 35 c1) applying a silver composition on the second side of the raw substrate,
 - d) applying a polymer composition on top of the second electrode region, to obtain a membrane, wherein the polymer composition comprises a hydrophobic polymer,

- e) cutting the raw substrate comprising the working electrode region, the second electrode region and the membrane to obtain the analyte sensor.

Process steps a) to e) may be carried out in the given order. However, it is also possible to carry out the steps in different orders. In particular, the order of steps b) and c) may be different. It is even possible, to first carry out step b1), then step c1) and thereafter step b2), for example. Further process steps are feasible. It is also possible to carry out at least one of process steps a) to e) more than once. For example, step c1) may be carried out more than once, so that more than one layer of the silver composition is obtained.

10

In step a) of the method for manufacturing the inventive analyte sensor, a raw substrate is provided.

Within the context of the present invention, the term "raw substrate" specifically may refer, without limitation, to any kind of material or combination of materials which is suitable to form a carrier layer to support the at least one working electrode and the at least one second electrode. From the raw substrate, the substrate of the inventive analyte sensor may be manufactured, for example, by cutting the raw substrate. In particular, the raw substrate may comprise an electrically insulating material. For the electrically insulating material, the embodiments and preferences described above for the electrically insulating material of the substrate hold true.

20

Thus, in a preferred embodiment the raw substrate comprises at least one electrically insulating material selected from the group consisting of an insulating epoxy resin, a polycarbonate, a polyester, a polyvinylchloride, a polyurethane, a polyether, a polyethylene, a polyamide, a polyimide, a polyacrylate, a polymethacrylate, a polytetrafluoroethylene or a copolymer thereof, and alumina.

25

A suitable polyester is for example polyethyleneterephthalate.

The raw substrate comprises a first side and a second side. To the person skilled in the art it is clear that the first side and the second side are different from one another.

30

In an embodiment the first side and the second side are positioned opposite each other. Therefore, in an embodiment the raw substrate comprises two opposing sides, the first side and the second side opposing the first side.

35

The raw substrate may be a flat substrate. Specifically the raw substrate may be flexible and/or deformable. Thus, as an example, the raw substrate may be a thin, flexible raw substrate. As an

example, the raw substrate may have a thickness of 50 μm to 1 mm, specifically a thickness of 80 μm to 500 μm , such as 110 μm to 250 μm .

5 The raw substrate may have a length which is preferably in the range from a few centimeters to several meters, such as for example in the range from 10 cm to 100 m.

The raw substrate may have a width which is preferably in the range from 2 centimeters (cm) to 8 cm.

10 In an embodiment, the raw substrate may comprise an electrically conductive material on at least one of the first and the second side, preferably on the first side and on the second side.

In an embodiment of the present invention, the raw substrate may be suitable to be used in a roll-to-roll process.

15 The raw substrate may be provided by any method known to the skilled person. For example, the raw substrate may be provided as a roll. This is particularly advantageous as the raw substrate may then be used in a roll-to-roll process.

20 In an embodiment, the raw substrate is cut into sheets before the working electrode region is prepared. The sheets may have any length, such as, for example, in the range from 100 mm to 300 mm.

In step b) a working electrode region is prepared on the first side of the raw substrate.

25 The working electrode region specifically comprises all components which form part of the at least one working electrode of the analyte sensor.

30 In step b1) the electrically conductive material is applied to the first side of the raw substrate. For the electrically conductive material the embodiments and preferences described above hold true.

The electrically conductive material can be applied to the first side of the raw substrate by any known method, for example via chemical vapor deposition (CVD), physical vapor deposition (PVD), or a wet-coating process. Wet-coating processes are known as such. A suitable wet-coating
35 process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing.

In step b2) a sensing material comprising at least one enzyme is applied at least partially on the electrically conductive material. For the sensing material and the at least one enzyme, the embodiments and preferences described above hold true.

- 5 The sensing material may be applied by any known method to the at least one electrically conductive material, for example by a wet-coating process. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing. After the wet-coating process, the layer of the sensing material may be further treated. Such treatments are for example drying treatments, curing treatments and/or laser ablation treatments. Such treatments are known as such.

- 15 The sensing material may be applied to the electrically conductive material so that it fully or partially covers the electrically conductive material, it may also overlap with the electrically conductive material. The sensing material may be applied to the electrically conductive material in any shape. For example in the shape of one or a plurality of lines, one or a plurality of dots, one or a plurality of strips. It is also possible to remove the sensing material partially from the at least one electrically conductive material after its application. Methods to remove the sensing material partially from the at least one electrically conductive material are known as such. For example, a portion of the sensing material may be irradiated by light, in particular by a laser, thereby removing the sensing material partially. It is also possible to irradiate a portion of the sensing material, thereby crosslinking the sensing material and afterwards washing the non-irradiated portion away.

In step c) a second electrode region is prepared on the second side of the raw substrate.

- 25 The second electrode region specifically comprises all components which form part of the at least one second electrode of the analyte sensor.

- 30 In step c1) a silver composition is applied to the second side of the raw substrate. In an embodiment, the silver composition is applied directly to the second side of the raw substrate. The silver composition may be applied to the second side of the raw substrate so that it covers the second side of the raw substrate at least partially. In another embodiment, the silver composition is applied at least partially to a second conductive trace.

- 35 The silver composition may be any composition known to the skilled person. In particular, the silver composition comprises silver. "Silver" within the context of the silver composition of the present invention not only encompasses elemental silver but also silver compounds. In particular,

the silver composition comprises Ag/AgCl and a polymer binder. For the polymer binder and for Ag/AgCl the preferences and embodiments described above hold true.

5 The silver composition may be applied to the second side of the raw substrate by any method known, for example by a wet-coating process. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing. After the wet-coating process, the layer of the silver composition may be further treated. Such treatments are for example drying treatments, curing treatments and/or laser ablation treatments. Such treatments are known as such.

10

In an embodiment, before step c1) a second conductive trace is applied to the raw substrate. For the second conductive trace the embodiments and preferences described above hold true. Thus, the second conductive trace may refer to a second electrically conductive material. For the second conductive material, the embodiments and preferences described above hold true.

15

The second electrically conductive material can be applied to the first side of the raw substrate by any known method, for example via chemical vapor deposition (CVD), physical vapor deposition (PVD), or a wet-coating process. Wet-coating processes are known as such. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing.

20

In step d) a polymer composition is applied on top of the second electrode region. For the polymer composition the embodiments and preferences described above hold true. A membrane is obtained. The membrane is obtained on the second electrode region. In particular, in step d) a membrane is formed on the second electrode region.

25

In an embodiment during the inventive process no polymer composition which comprises a hydrophobic polymer is applied to the first side of the substrate.

30 The polymer composition may be applied on top of the second electrode region by any method known, for example by a wet-coating process. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing. After the wet-coating process, the layer of the polymer composition may be further treated. Such treatments are for example drying treatments, curing treatments and/or laser ablation treatments. Such treatments are known as such.

35

In an embodiment, the polymer composition, after its application to the second side of the raw substrate, preferably the membrane which is obtained in step d) may be irradiated by a laser to form holes. For the holes, the preferences and embodiments described above for the holes of the membrane hold true.

5

In step e) the raw substrate comprising the working electrode region, the second electrode region and the membrane is cut to obtain the analyte sensor.

The raw substrate is preferably cut along its width, so that strips are formed. These strips may correspond to the analyte sensor. It is also possible that, before or after the raw substrate is cut along its width that the raw substrate is cut at least once along its length.

10

The raw substrate is preferably cut with a laser.

Thus, preferably in the method for manufacturing an analyte sensor the cutting in step e) comprises laser-cutting.

15

When cutting the raw substrate in step e), holes may be formed in the cutting region of the membrane.

20

Further process steps may be carried out. For example, in a step f) a flux limiting membrane may be applied.

Thus, in an embodiment of the inventive method the following step f) is carried out:

25

f) applying a flux limiting membrane to the analyte sensor obtained in step e) to obtain a covered analyte sensor.

For the flux limiting membrane, the preferences and embodiments described above hold true. In particular, the flux limiting membrane, preferably the at least one polymeric material comprised in the flux limiting membrane, may for example be applied by a wet-coating process. A suitable wet-coating process is for example selected from the group consisting of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and screen printing.

30

For example, in a step g) a biocompatibility membrane may be applied.

35

Thus, in an embodiment of the inventive method, the following step g) is carried out:

g) applying a biocompatibility membrane to the analyte sensor obtained in step e).

If step f) is carried out, then the biocompatibility membrane is typically applied to the covered
5 analyte sensor obtained in step f)

Thus, in case step f) is carried out, in an embodiment the following step g) is carried out:

g) applying a biocompatibility membrane to the covered analyte sensor obtained in step f).
10

For the biocompatibility membrane, the preferences and embodiments described above hold true.
In particular, the biocompatibility membrane usually consists of a biocompatible material. Thus,
preferable, in step g) a biocompatible material is applied. The biocompatibility membrane,
preferably the biocompatible material may be applied by any process known, in particular by a wet-
15 coating process. A suitable wet-coating process is for example selected from the group consisting
of spin-coating, spray-coating, doctor-blading, printing, dispensing, slot-coating, dip coating and
screen printing.

Another object of the present invention is, therefore, also an analyte sensor obtainable by the
20 inventive method for manufacturing an analyte sensor.

A further object of the present invention is an analyte sensor system comprising:

- the inventive analyte sensor and
- an electronics unit, the electronics unit being electronically connected to the analyte
25 sensor.

For the analyte sensor comprised in the analyte sensor system, the embodiments and preferences
described above for the inventive analyte sensor hold true.

30 The term “electronics unit” as used herein is a broad term and is to be given its ordinary and
customary meaning to a person of ordinary skill in the art and is not to be limited to a special or
customized meaning. The term specifically may refer, without limitation, to a unit, such as a unit
which may be handled as a single piece, which is configured for performing at least one electronic
function. Specifically, the electronics unit may have at least one interface for being connected to
35 the analyte sensor, wherein the electronics unit may provide at least one electronic function
interacting with the analyte sensor, such as at least one measurement function. The electronics unit
specifically may be configured for measuring at least one voltage and/or for measuring at least one

current, thereby interacting with the analyte sensor. The electronics unit may further comprise at least one integrated circuit, such as a processor and/or a battery. The term "processor" as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term

5 specifically may refer, without limitation, to an arbitrary logic circuitry configured for performing basic operations of a computer or system, and/or, generally, to a device which is configured for performing calculations or logic operations. In particular, the processor may be configured for processing an electronic signal, such as a current or a voltage, specifically an electronic signal from the analyte sensor. Specifically, the processor may be or may comprise a microcontroller unit

10 (MCU). Additionally or alternatively, the processor may be or may comprise a microprocessor, thus specifically the processor's elements may be contained in one single integrated circuitry (IC) chip. Additionally or alternatively, the processor may be or may comprise one or more application-specific integrated circuits (ASICs) and/or one or more field-programmable gate arrays (FPGAs) or the like. The processor specifically may be configured, such as by software programming, for

15 performing one or more evaluation operations. Thus, the processor may be configured for processing and/or evaluating the electronic signal from the analyte sensor and, for example, outputting a signal indicating the analyte concentration measured by the analyte sensor. The electronics unit further may comprise at least one measuring device for measuring at least one of a voltage and a current, such as a potentiostat. Further, the electronics unit may comprise a

20 microcontroller, specifically being configured for controlling one or more electronic functions of the electronics unit.

The electronics unit specifically may comprise at least one electronics unit housing, wherein the analyte sensor, e.g. with a proximal end and/or an end providing electrical contacts for contacting

25 the analyte sensor, may protrude into the electronics unit housing and may be electrically connected with at least one electronic component within the electronics unit housing. As an example, the proximal end and/or at least one contact portion of the analyte sensor may protrude into the electronics unit housing and, therein, may be electrically connected to at least one electronic component, such as to at least one printed circuit board and/or at least one contact

30 portion of the electronics unit, e.g. by one or more of a soldering connection, a bonding connection, a plug, a clamping connection or the like. The electronics unit specifically may be used and/or configured as a transmitter for transmitting measurement data to at least one external device, such as to at least one receiver, e.g. wirelessly.

35 The electronics unit is electronically connected to the analyte sensor. Thus, an electrical connection exists between the analyte sensor and the electronics unit. The electronics unit comprised in the analyte sensor system is in contact with the analyte sensor. For example, the conductive trace and

the second conductive trace of the analyte sensor may each form an electrical connection with the electronics unit. Typically, the analyte sensor comprises the contact portion at a proximal end and the working electrode and the second electrode at a distal end. Thus, an electrical signal, such as an electrical current and/or an electric voltage, may be transmitted via the electronic connection from the analyte sensor to the electronics unit. Via the electrical connection, the electronics unit may interact with the analyte sensor for performing at least one electrochemical measurement. The electrical connection specifically, as outlined above, may be established by at least one connection portion of the analyte sensor protruding into a housing of the electronics unit.

10 In the Figures

Figure 1: shows experimental results

Examples

15

The following examples serve to illustrate the invention. They must not be interpreted as limiting with regard to the scope of protection.

For in vivo tests analyte sensors were prepared, the analyte sensors comprised the following:

- 20
- substrate: PET, thickness of 130 μm
 - working electrode:
 - electrically conductive material: layer of gold (100 nm thickness) with a layer of carbon paste on top
 - enzyme: glucose oxidase comprised in sensing chemistry (Os-complex modified polymer)
 - 25 - second electrode: combined counter/reference electrode
 - electrically conductive material: layer of gold (100 nm thickness)
 - silver: Ag/AgCl paste

30 A membrane comprising a hydrophobic polymer (hydrophobic thermoplastic polyurethane) was located on top of the second electrode. The analyte sensors were laser cut. Holes were formed in the hydrophobic polymer of the analyte sensor depending on the laser cutting conditions of the cutting. A flux limiting membrane and a biocompatibility membrane covered the sensors.

Analyte sensors with the following total area of holes in the hydrophobic polymer were prepared:

35

Sensor 1: 0 mm²

Sensor 2: 0.03 mm²

Sensor 3: 0.1 mm²
Sensor 4: 0.32 mm²

5 The analyte sensors were implanted in vivo in the same subject and the current measured over a period of eight days. In parallel regular blood glucose values (BG values) were measured with a BG meter using finger pricking. Figure 1 shows the normalized current for the measurements with Sensor 2, Sensor 3 and Sensor 4. 0 on the x-axis shows the start of the measurement time after a run-in time of about 1 hour. The measurement curve for Sensor 1 is not shown as no current could be obtained.

10

Sensor 2 shows from the beginning of the measurement a sufficiently high current which remains constant throughout the whole measurement time (eight days). There are some peaks which indicate high and low glucose values during the measurement time. These peaks correspond to the peaks found in the regular blood glucose value measurements. Thus, the measurement with sensor 15 2 is particularly reliable and stable even over a longer time period. Further it allows calibration with the regular blood glucose value measurements

20 Sensor 3 has a significantly longer run-in time, during which the current did not correlate with the BG values. It can be seen from figure 1 that the expected current is only reached after one day of measurement. Thereafter, the measurement is comparable in its reliability and stability with the one of sensor 2.

25 Sensor 4 exhibited an even longer run-in time, during which the current was significantly lower than expected. Though there are some peaks in the current visible, they did not correlate with the BG values and the sensitivity of the sensor (ratio of the current to the BG values) was too low and instable. After day seven, the current was significantly higher and correlated with the BG values and the sensitivity increased.

30

Claims

1. An analyte sensor comprising
 - 5 - a substrate comprising a first side and a second side;
 - at least one working electrode positioned on the first side of the substrate, the at least one working electrode comprising:
 - at least one electrically conductive material and
 - at least one enzyme;
 - 10 - at least one second electrode positioned on the second side of the substrate, the at least one second electrode comprising silver;
 - a membrane comprising a polymer composition which comprises a hydrophobic polymer, wherein the membrane is located on top of the at least one second electrode, wherein the membrane comprises holes, wherein the total area of holes has a size of at most 0.15 mm².
- 15 2. The analyte sensor according to claim 1, wherein the analyte sensor is an implantable sensor.
3. The analyte sensor according to claim 1 or 2, wherein the at least one second electrode is selected from the group consisting of a counter electrode, a reference electrode and a
- 20 combined counter/reference electrode.
4. The analyte sensor according to any one of claims 1 to 3, wherein the first side and the second side of the substrate are positioned opposite each other.
- 25 5. The analyte sensor according to any one of claims 1 to 4, wherein the at least one second electrode comprises Ag/AgCl.
6. The analyte sensor according to claim 5, wherein the load of AgCl of the at least one second electrode is in the range from 20 µg to 150 µg.
- 30 7. The analyte sensor according to any one of claims 1 to 6, wherein the polymer composition comprises a hydrophobic thermoplastic polyurethane.
8. The analyte sensor according to any one of claims 1 to 7, wherein the hydrophobic polymer
- 35 has a glass transition temperature, wherein the glass transition temperature is in the range from -100 °C to 0 °C.

9. The analyte sensor according to any one of claims 1 to 8, wherein the hydrophobic polymer has a water uptake of less than 1 % by weight, based on the total weight of the hydrophobic polymer.
- 5 10. The analyte sensor according to any one of claims 1 to 9, wherein the at least one working electrode is free of the membrane comprising the polymer composition which comprises the hydrophobic polymer.
11. A method for manufacturing an analyte sensor, the method comprising the steps:
- 10 a) providing a raw substrate which comprises a first side and a second side,
b) preparing a working electrode region on the first side of the raw substrate, the preparing of the working electrode region comprising the steps:
b1) applying an electrically conductive material to the first side of the raw substrate,
b2) applying a sensing material comprising at least one enzyme at least partially on the
15 electrically conductive material,
c) preparing a second electrode region on the second side of the raw substrate, the preparing of the second electrode region comprising the steps:
c1) applying a silver composition on the second side of the raw substrate,
d) applying a polymer composition on top of the second electrode region, to obtain a
20 membrane, wherein the polymer composition comprises a hydrophobic polymer,
e) cutting the raw substrate comprising the working electrode region, the second electrode region and the membrane to obtain the analyte sensor.
12. The method according to claim 11, wherein the cutting in step e) comprises laser-cutting.
- 25 13. An analyte sensor obtainable by a method according to claim 11 or 12.
14. An analyte sensor system comprising
- 30 - an analyte sensor according to any one of claims 1 to 10 or 13,
- an electronics unit, the electronics unit being configured to electronically connect to the analyte sensor.