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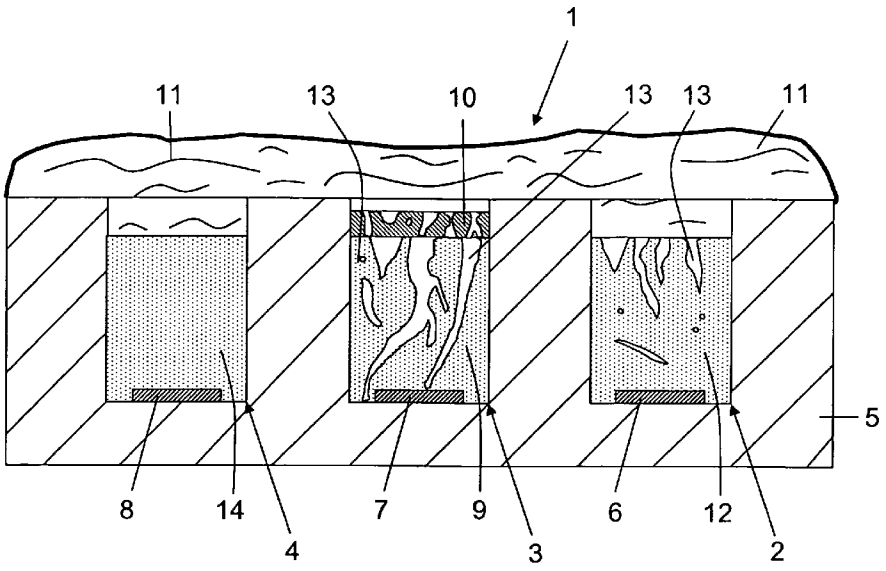
(54) Title: AMPEROMETRIC SENSOR AND METHOD FOR ITS MANUFACTURING



(57) Abstract: The invention refers to an amperometric sensor (1) configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said sensor (1) comprising a counter electrode (2) and a working electrode (3), said working electrode (3) comprising a sensing layer (9) which is permeable for water and arranged on a support member (5) adjacent to a contact pad (7), said sensing layer (9) comprising an immobilized enzyme capable of acting catalytically in the presence of the analyte to cause an electrical signal, the sensing layer (9) having an upper surface facing the body fluid and a lower surface facing away from the body fluid. According to the invention the immobilized enzyme is distributed in the sensing layer (9) in such a way that the enzyme concentration in the middle between its upper and lower surface is at least as high as on the upper surface of the sensing layer (9).

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Amperometric sensor and method for its manufacturing

The invention refers to an amperometric sensor configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said sensor comprising a counter electrode and a working electrode, said working electrode comprising a sensing layer which is permeable for water and arranged on a contact pad, said sensing layer comprising an immobilized enzyme capable of acting catalytically in the presence of the analyte to cause an electrical signal, the sensing layer having a lower surface facing the contact pad and an upper surface facing away from the contact pad. Such a sensor is known from EP 0247850 B1.

Implantable sensors for the in vivo-measurement of medically important analytes like glucose or lactate are based on electrochemical enzymatic detection of the analyte. The most common approach is the use of an oxidase to oxidize an analyte, e. g. glucose, with subsequent reduction of oxygen to hydrogen peroxide and amperometric detection of the hydrogen peroxide by a working electrode of the sensor. Another approach in the field of in-vivo sensing bypasses the use of oxygen/peroxide as a mediator couple by employing synthetic redox mediators for glucose conversion without oxygen. In that case, synthetic redox mediators are built into the sensing element. An example for this approach utilizes poly(biimidizyl)osmium complexes as redox mediators in conjunction with enzyme as described by Feldmann et al. in Diabetes Technology and Therapeutics, 5, 769 (2003).

Despite intensive research and development efforts there are at present no implantable sensors available which measure medically important analytes like glucose reliably over extended periods of time.

It is an object of the present invention to provide a way to improve the reliability and longevity of amperometric sensors for in vivo-measurements of an analyte concentration in a body fluid.

According to the invention this object is achieved by an amperometric sensor configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said sensor comprising a counter electrode and a working electrode, said working electrode comprising a sensing layer which is permeable for water and arranged on a support member adjacent to a contact pad, said sensing layer comprising an immobilized enzyme capable of acting catalytically in the presence of the analyte to cause an electrical signal, the sensing layer having an upper surface facing the body fluid and a lower surface facing away from the body fluid, characterized in that the immobilized enzyme is distributed in the sensing layer in such a way that the enzyme concentration in the middle between its upper and lower surface is at least as high as on the upper surface of the sensing layer.

In a planar configuration, the contact pad can be placed directly under the sensing layer (or the sensing layer is arranged on the contact pad) both having identical surface areas. In another embodiment, the contact pad can be made smaller or larger than the sensing layer. In still another embodiment, the contact pad can be partly displaced from the area covered by the sensing layer, so that only a fraction of the sensing layer is in direct contact with the pad. For other arrangements, the contact pad can be placed on one of the sides of the sensing layer. All these options are summarized by the term "the sensing layer adjacent to the contact pad". It is understood that this holds likewise for the other electrodes.

It has been found that measurements of implanted amperometric sensors are often adversely affected by a low oxygen concentration in

subcutaneous tissue surrounding the sensor. This problem seems to be especially pronounced in the case of enzymatic sensors which rely on an oxidase, for example a glucose oxidase, as the immobilized enzyme in the sensing layer, as such sensors cause an electrical measurement signal by oxidizing the analyte. In principle, the strength of the measurement signal created by such sensors depends on the amount of enzyme, analyte and oxygen present. If the oxygen concentration is sufficiently high, the response of a given sensor with a defined enzyme loading reflects the concentration of the analyte in the vicinity of the sensor and is, in ideal cases, proportional to it. However, if the oxygen concentration is too low, fewer analyte molecules are oxidized and consequently a weaker electrical signal is produced compared to a sensor operating under oxygen-saturated conditions.

Lowering the enzyme loading of a sensor lowers the critical oxygen concentration, at which saturation is achieved, but also reduces the signal to noise ratio as a smaller measurement signal is created. Hence, lowering the enzyme loading is not sufficient to solve the problem.

An amperometric sensor according to the present invention deals with the problem of low oxygen concentrations in subcutaneous tissue by means of the sensing layer which comprises the immobilized enzyme which is distributed in the sensing layer in such a way that the enzyme concentration in the middle between the upper and lower surface is at least as high as on the upper surface of the sensing layer.

Accordingly, only a relatively small fraction of the enzyme molecules contained in the sensing layer is active on the upper surface of the sensing layer. As a consequence, a relatively low concentration of oxygen is sufficient to saturate the surface of the sensing layer with oxygen. The structure of the sensing layer allows analyte molecules to diffuse into the sensing layer and interact with enzyme molecules further away from the surface which are surrounded by their own reservoir of oxygen molecules. Thus, the electrical signal of a sensor according to the present invention is created not only within a small surface layer but rather within an

extended volume which lowers the oxygen density (oxygen concentration) at which saturation of the sensor is achieved. Consequently, saturation of enzyme with oxygen can be achieved at lower oxygen concentrations without lowering the signal to noise ratio of the measurement signal of the sensor.

Amperometric sensors with a porous sensing layer have been described in EP 0247850 B1. However, the enzyme was supplied to the known sensor only after the porous layer had already been prepared. Therefore, the enzyme concentration of the sensor described in EP 0247850 B1 is highest on the upper surface of the sensing layer and decreases strongly with increasing distance from the surface. As a consequence, the major part of the electrical signal of such a sensor is created on the surface of the sensing layer, i.e. in a relatively small volume so that correspondingly higher oxygen concentrations are required for precise measurements.

A distribution of enzyme throughout the sensing layer according to the present invention, especially a homogenous concentration, can be most easily achieved by mixing enzyme into a paste, preferably a paste comprising carbon particles and a binder, and applying this mixture onto a contact pad to provide the sensing layer of a working electrode. In some cases it is advantageous to use a surface-active agent such as a detergent or a hydrophilic polymer to aid the dispersion of the enzyme within the paste. In this way an equal distribution of enzyme molecules throughout the sensing layer can be achieved. The object of the invention is therefore also achieved by a method for manufacturing an amperometric sensor configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said method comprising the following steps: mixing carbon particles, enzyme and a polymeric binder to create a paste; applying that paste adjacent to a contact pad onto a support member and hardening that paste into a porous sensing layer.

Further details and advantages of the present invention are illustrated in the following on the basis of an exemplary embodiment making reference

to the attached figures. The features illustrated therein can be used individually or in combination to define the invention. In the figures:

Fig. 1 shows a first exemplary embodiment of a sensor according to the present invention in a cross section view.

Fig. 2 shows a functional characteristic of the sensor according to figure 1 from an in vitro measurement.

Fig. 3 shows measurement data of the sensor according to figure 1 measured in a biomatrix.

Fig. 4 shows the dependence of the sensor current on a diffusion barrier covering the sensing layer of sensors F to J, but not sensors A to E.

Fig. 5 shows a second exemplary embodiment of a sensor according to the present invention in a cross section view.

Figure 1 shows schematically a first embodiment of an amperometric sensor 1 configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid of a human or animal. For better illustration of some details the figure 1 is not to scale.

The sensor 1 comprises a counter electrode 2, a working electrode 3 and a reference electrode 4 which are arranged on a support member 5 made of a plastic material, especially polyimide. Each electrode 2, 3, 4 comprises a contact pad 6, 7, 8 which is provided as an electrically conductive film, e.g. a metallic film, in particular a gold film, with a thickness of preferably 50 nm to 150 nm. It is also possible to make the contact pads 6, 7, 8 from other metals, in particular palladium, or as a multilayer film of different metals. For example, a thin film, less than 20 nm, of titanium covering the support member 5 can be covered with a second film of gold with a thickness of 50 to 130 nm, thus forming the contact pads 6, 7, 8. As an alternative, the contact pads 6, 7, 8 can be formed as an electrically conductive polymer film, e.g. from a conductive polymer paste, for example by screen-printing or by dispensing which

leads to thicker contact pads 6, 7, 8. Instead of separate counter and reference electrodes 3, 4 a combined counter/reference electrode may also be used. One example of a suitable counter/reference electrode is a silver/silver-chloride electrode. As such counter and/or reference electrodes are commonly used no further description is necessary.

The working electrode 3 further comprises a sensing layer 9 which is permeable for water and arranged adjacent to the contact pad 7 of the working electrode 3. The sensing layer 9 comprises immobilized enzyme capable of acting catalytically in the presence of the analyte to cause an electrical signal. In the present example an oxidase, particularly a glucose oxidase is used as enzyme to measure glucose as an analyte in a human body fluid, like interstitial fluid or blood.

The sensing layer 9 was applied as a paste onto the support member 5 to cover the contact pad 7 of the working electrode 2. That paste was made by mixing carbon particles, enzyme and a polymeric binder. In this way the immobilized enzyme is distributed equally throughout the sensing layer 9. A homogeneous enzyme distribution throughout the sensing layer 9 is advantageous. Hence, the enzyme concentration should differ by less than 20%, especially less than 10%, between the upper surface and the lower surface of the sensing layer 9. As the analyte can diffuse into the porous sensing layer 9 the electrical measurement signal is created not just in the sensing layers 9 upper surface which faces away from the contact pad 7 but rather in an extended volume. Therefore, rather low oxygen concentrations are sufficient to saturate the sensor 1 with oxygen and enable precise measurements.

In preferred embodiments the sensing layer 9 is flat. Preferably the sensing layer 9 is electrically conductive, wherein the electrical conductivity of the sensing layer 9 is at least $1\ \Omega^{-1}\ cm^{-1}$. By this the advantage is achieved that every place in the sensing layer 9 at which the enzymatic reaction of the analyte takes places acts as a tiny electrode, at which immediately the product of the enzymatic reaction can be reduced or oxidized. In this manner these places are used as cathode or anode,

depending on the sign of the electrical potential applied. Consequently the sensing layer 9 in the porous structure comprises a huge number of tiny cathodes or anodes. As a result of this there is no need for the product of the enzymatic reaction to advance through the bulk of the sensing layer 9 which would result in a loss a signal height. An electrically conductive embodiment of the sensing layer 9 therefore has the result of an increased signal height.

The sensing layer 9 of the example shown has a thickness of 30 µm. Generally the sensing layer 9 should have a thickness of at least 5 µm, preferably at least 10 µm, in order to provide a sufficiently large volume for the creation of the electrical measurement signal. A thickness of the sensing layer 9 of more than 100 µm does not provide additional benefits. A thickness of 20 µm to 70 µm is preferred. The sensing layer 9 is arranged in a depression of the support member 5. In this way it is somewhat protected by lateral walls of the support member 5 from damages during the implantation process. Furthermore, the lateral surfaces of the sensing layer 9 can be connected to the support member 5 and thereby ensure that analyte molecules can diffuse only through the sensing layer's 9 upper surface into the sensing layer 9. Of course, the lateral surfaces can be made impervious to water by different means as well. The sensing layer 9 can have lateral surfaces which are impervious for the body fluid.

In similar fashion the contact pads 6, 8 of the counter electrode 2 and the reference electrode 4 are covered with water-permeable layers 12, 14 which were also applied as paste. Of course, the layers 12, 14 of the counter electrode 2 and of the reference electrode 4 contain no enzyme. Like the sensing layer 9, layers 12 and 14 may also comprise carbon particles and a polymeric binder. Whereas porosity enhancing particles 13 like carbon nanotubes have been added to the pastes for the sensing layer 9 and the layer 12, such porosity enhancing particles 13 provide no benefit for the highly conductive layer 14 of the reference electrode 4 and were therefore not added.

As enzyme is distributed throughout the whole sensing layer 9, oxygen saturation can be maintained even if much higher analyte concentrations are present at the upper surface of the sensing layer 9 than is feasible for known sensors. The sensing layer of sensors according to prior art is usually covered by a diffusion barrier which hinders analyte diffusion to such an extent that the analyte concentration at the upper surface is typically about 100 times lower than in body fluid surrounding the sensor.

The sensing layer 9 of the sensor 1 of the embodiment of the invention is covered by a diffusion barrier which hinders diffusion of analyte molecules only to such an extent that after implantation into the living body of a human or animal the analyte concentration at the upper surface of the sensing layer 9 is at most ten times lower than in the body fluid surrounding the implanted sensor 1, especially at most five times lower, preferably at most three times lower. In the example shown, the diffusion barrier comprises several distinct layers 10, 11 contributing to the diffusion resistance of the diffusion barrier against diffusion of analyte molecules.

The diffusion barrier is permeable for the analyte and prevents enzyme from leaking out of the sensing layer 9. In the example shown, the diffusion barrier comprises as a first layer an electrically conductive enzyme-free layer 10 which comprises carbon particles and a polymeric binder and has a thickness of less than a third of the thickness of the sensing layer 9. Usually it is about 1 µm to 3 µm thick. Like the sensing layer 9 the enzyme-free layer 10 was applied as a paste. That paste differs from the paste of the sensing layer 9 only in that no enzyme was added to it.

The diffusion barrier also comprises a layer 11 which prevents large molecules from clogging pores of the sensing layer 9. The layer 9 may be a dialysis layer which can be provided as a membrane made of cellulose and/or a polymer material. Such a dialysis layer is also an enzyme-free layer and may be applied directly on top of the sensing layer 9 or, as shown in figure 1, on top of the electrically conductive enzyme-free layer

10. It is advantageous, if such a dialysis layer hinders analyte diffusion as little as possible. Preferably, the layer 11 has an effective diffusion coefficient D_{eff} for the analyte which is at most ten times lower than the diffusion coefficient D of the analyte in water, especially at most five times lower than the diffusion coefficient D of the analyte in water. A dialysis layer can be applied as a solid film or applied as a polymer solution which hardens into a dialysis membrane in-situ.

Dialysis membranes are often characterized by their molecular weight cut off (MWCO) which depends on the pore size. The MWCO describes the molecular weight at which a compound will be 90 % retained following of a night (17-hour) dialysis. The dialysis layer of the example shown has a MWCO of less than 10 kDalton, preferably of less than 7 kD, especially of less than 5 kD. It has to be understood that MWCOs stated for dialysis layers apply strictly only to globular molecules such as most proteins. More linear molecules may be able to pass through the pores of a dialysis layer, even if their molecular weight exceeds the stated MWCO.

Instead of or in addition to a dialysis membrane the diffusion barrier may also comprise a polymer layer made of a polymer having a zwitterionic structure to protect the sensing layer 9 and any porous layer 10 from ingression of proteins. A zwitterionic structure enables the rapid uptake of polar protic solvents, in particular water, and such analytes as glucose dissolved within. Hence, polymers having a zwitterionic structure attached to a polymeric backbone are impermeable for proteins but hinder diffusion of analytes like glucose very little. A well-known example for such a polymer is poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butylmethacrylat) (MPC for short). The MPC polymer layer 11 is applied as a polymer solution comprising ethanol or distilled water and at least 5 wt.% MPC, especially at least 10 wt.% MPC.

The diffusion barrier and especially the polymer layer 11, which it comprises, protect the sensor 1 from mechanical damage during the implantation process, prevent enzyme from leaking out of the sensing layer 9 into surrounding tissue, where it might be harmful, and prevents

large molecules from clogging pores of the sensing layer 9. It is possible to mix a polymer having a zwitterionic structure like MPC with another polymer, for example polyurethane or typical constituents of the above-mentioned dialyse membranes, in order to tune physical properties of the polymer layer 11.

It is also possible to tune physical properties, such as the permeability for analytes, of the layer 11, if this contains a co-polymer having constituents of different hydrophilicity, by varying the relative amount of each constituent in the co-polymer. In the case of MPC it is possible to increase the relative amount of 2-methacryloyloxyethyl phosphorylcholine vs that of butylmethacrylat from 30:70% to 50:50% yielding a co-polymer with higher permeability towards polar protic solvents or glucose. Another way to increase permeability for polar protic solvent or glucose is to change the hydrophobic backbone of the co-polymer into a more hydrophilic entity. This also applies for other water-soluble analytes.

The sensing layer 9 of the example shown in figure 1 contains porous particles 13 to increase its porosity and thereby ease diffusion of analyte molecules into the sensing layer 9. Porous particles 13 in this respect are particles which have voids to adsorb water molecules. These porous particles 13 are added to the paste from which the sensing layer 9 is formed and cause voids through which analyte molecules and water may pass. The porous particles 13 are bound with other particles of the paste by the polymeric binder. Carbon nanotubes are an especially useful additive to increase the porosity of the sensing layer as they tend to form clews, which are only partially filled with carbon particles and binder, and also increase the electrical conductivity of the sensing layer. Silica particles may also be used as porous particles 13 to increase the porosity of the sensing layer 9.

If silica or similar porous particles 13 are used, it is advantageous to use material with a particle size distribution such that the maximum particle size is less than the thickness of the sensing layer 9. To be most effective, the porous particles 13 should measure at least 1 µm, especially at least

5 µm. Considering a sensing layer 9 thickness around 20 µm to 50 µm, silica FK 320 from Degussa provides adequate particle size, up to 15 µm. Typically, less than 10% of this material are mixed into the paste, preferably less than 5%.

It is important to provide electrical conductivity throughout the sensing layer 9, so that at each spot of the porous matrix, where a product molecule is generated from the enzymatic reaction, this molecule is directly oxidized or reduced by application of the appropriate voltage without the need for extended diffusion of this molecule to a distant site. Under these circumstances the porous and permeable sensing layer 9 is capable of electrolyzing the analyte substantially throughout the entire layer.

Whatever means for increasing the porosity is used, the mixing of the enzyme with the paste will lead to a fraction of enzyme molecules being accessible to the analyte, either on the upper surface of the sensing layer 9, or at the channels in the vicinity of the additive particles within the sensing layer 9. The enzyme is immobilized by adsorption and entrapment in the working electrode 3. Entrapment depends not only on the sensing layer 9 but also on properties of the diffusion barrier, i.e. the layer 11, and of the optional enzyme-free layer 10. It is understood that in order to maintain the ideal distribution of enzyme within the working electrode, contact with solvent (water) should not lead to massive detachment of enzyme from the matrix and subsequent migration of enzyme molecules. Enzyme immobilization in the sensing layer 9 can be enhanced by cross-linking. Especially advantageous are enzyme molecules which are cross-linked as a chain. If these chains are too long, the enzyme is less effective. It is therefore preferred that on average three to ten, especially four to eight, enzyme molecules are linked together. A chain length of five to seven enzyme molecules seems to be most advantageous.

It is possible to add a cross-linking agent, i.e. glutaraldehyd solution, to the paste before drying. However, it is preferable to mix an already cross-

linked enzyme into the paste. It is advantageous to use enzyme which forms a complex with a hydrophilic partner. After being mixed into a paste which is less hydrophilic or even hydrophobic, as can be achieved by mixing carbon particles with suitable binders, the cross-linked enzyme sits in a local hydrophilic environment which contributes to its stability. An additional advantage of a cross-linked enzyme with a hydrophilic partner is that it enhances migration of hydrated analyte molecules towards the enzyme. Thus the wetting of the sensing layer 9 is accelerated which shortens the wet-up time of the sensor after implantation. As a specific example, glucose oxidase cross-linked with dextrane from Roche Diagnostics (Penzberg, Germany, Ident-No. 1485938001) has been found to have such a content of enzyme (approximately 16%) that enough activity (20 to 40 U/mg lyophylisate) can be preserved. Due to the high degree of hydrophilic dextrane in the complex, the aforementioned advantages can be exploited.

By mixing already cross-linked enzyme with the sensing layer paste containing carbon nanotubes, the trait of the carbon nanotubes to wind up and form clews, which act as macroporous cage structures, is supported by the larger enzyme-dextrane chains, in particular by their aggregation. As a consequence, the cross-linking enzyme will assist in the formation of porous structures of the sensing layer 9.

The sensing layer 9 of the example shown comprises carbon particles with an average size of less than 1 µm, polymeric binder, enzyme and carbon nanotubes as porous particles 13. The porous particles 13 are most effective to increase the porosity of the sensing layer 9 if they are significantly larger than the carbon particles. In the example shown, the porous particles 13 measure at least 1 µm, especially at least 5 µm, on average. Typically the sensing layer 9 comprises 50 wt.% to 70 wt.% polymeric binder, 20 wt% to 40 wt.% carbon particles and up to about 20 wt.%, preferably 1 wt.% to 10 wt.%, porous particles 13 like carbon nanotubes or silica. Carbon nanotubes are an especially advantageous additive as they increase both the porosity and the electrical conductivity of the sensing layer 9. In the embodiment shown schematically in figure 1

multiwall carbon nanotubes (research grade, purity > 95%) by NanoLab, Newton, MA, of length 5 µm to 20 µm and an average outer diameter of 25 nm to 35 nm have been used. The binder is a thermoplastic resin, e.g. on the basis of an epoxy resin or on the basis of polyvinyl chloride (PVC) / polyvinyl alcohol (PVA). Resins on the basis of a fluor carbon resin, particularly polytetrafluoroethylene, or of polystyrene, may also be used as binders. In the case of PVC/PVA binders, the use of additives like silicone oil can help to adjust the viscosity of the paste.

In this way the sensing layer 9 of the sensor 1 shown in figure 1 is adapted and arranged in such a way that in operation after implantation the analyte concentration in the sensing layer 9 is highest at the upper surface, decreases with increasing distance from the upper surface, and is zero at the lower surface which is the furthest point from the analyte-containing body fluid and which touches the contact pad 7. The enzyme loading of the sensing layer 9, i.e. the amount of the enzyme immobilized therein, should be chosen with respect to the porosity and water-permeability of the sensing layer 9.

An important parameter in this respect is the effective diffusion coefficient D_{eff} of the sensing layer 9. The effective diffusion coefficient D_{eff} characterizes the diffusion of the analyte in the sensing layer 9 and depends on the pore volume ε and the tortuosity τ of the sensing layer 9. Generally, the effective diffusion coefficient D_{eff} can be described as $D_{eff}=D\cdot\varepsilon/\tau$, wherein D is the diffusion coefficient of the analyte in water. The quotient τ/ε is also called hindrance H. In the example shown H is between 10 and 1000, especially between 50 and 500.

Another important parameter in this respect is the enzyme loading parameter α which can be described as $\alpha=(V_{max}\cdot d)/(K_M\cdot D)$ wherein V_{max} is the enzyme activity density which determines the maximum speed of analyte conversion, K_M the Michaelis Menten constant of the enzyme, d the thickness of the sensing layer and D the diffusion coefficient of the analyte in water. Preferably the ratio of the effective diffusion coefficient

D_{eff} in the sensing layer 9 and the enzyme loading parameter α is in the range of 10 to 200.

Figure 2 shows the functional characteristic of the sensor described above. The layer 11 has been made from MPC (Lipidure CM 5206, NOF Corp. Japan) by dispensing a 10% solution of MPC in ethanol/water on the electrodes. The measurement current I in nA is plotted versus glucose concentration g in mg/dl. The data shown in figure 2 were measured in-vitro in aqueous glucose solution. As can be seen, no saturation at higher glucose concentrations is observed.

Figure 3 shows for comparison measurement currents I_A and I_B in nA. Currents I_A were measured in-vitro, currents I_B by a sensor in a biomatrix, both at a temperature T = 35 °C, after the sensors had been equilibrating in the respective medium for 12 hours. Every data point shown belongs thus to a biomatrix measurement and an aqueous glucose solution measurement at identical glucose concentration. The biomatrix used consists of stabilized blood plasma, to which glucose was added in order to obtain the desired glucose concentration. Sensor currents measured in the biomatrix and sensor currents measured in aqueous glucose solution show excellent agreement.

The result is particular noteworthy and demonstrates the profound effects arising from the sensor layout of the embodiment of the present invention. In general, it is expected, that exposure of a sensor 1 to a biomatrix ensures the deposition of proteins, peptides or fibrin on the sensor surface. This process affects the permeability for analytes or water of an outer layer, such as layer 11. In a conventional sensor layout, this layer restricts diffusion of analyte to the sensing layer so that a permeability decrease results in a weaker measurement signal.

However, the signal height of the described sensor 1 is not affected by exposure to a biomatrix, as seen in figure 3, since the diffusion of analyte through the layer 11 is not the rate-limiting step in the generation of the

signal. Therefore any permeability alteration has very little effect on the signal for the sensor 1 described above.

This advantage of the invention is not limited to enzymes using oxygen as co-substrate in the catalytic reaction. The enzyme may be a dehydrogenase as well. For example, a glucose dehydrogenase which does not use oxygen as co-substrate can be distributed within the sensing layer 9. Known dehydrogenases include certain molecules as cofactors for the oxidation of glucose, for example pyrroloquinoline quinone (PQQ), or flavin adenine dinucleotide (FAD) or nicotinamid adenine dinucleotide (NAD), see EP 1 661 516 A1. Any of these dehydrogenases can be used in the sensing layer 9 instead of an oxidase.

Figure 4 shows the sensor current I in nA, which was measured by sensors A to J in phosphate-buffered aqueous glucose solution of different concentrations. Sensor currents measured at a glucose concentration of 360 mg/dl are depicted by triangles (▲). Sensor currents measured at a glucose concentration of 180 mg/dl are depicted by squares (■). Sensor currents at a glucose concentration of zero are depicted by diamonds (♦).

Sensors A to J differ only with respect to the diffusion barrier applied on top of the sensing layer 9. At sensors A to E a diffusion barrier is lacking, i.e. the sensing layer 9 is in direct contact with the aqueous glucose solution to be measured. Sensors F to J comprise a diffusion barrier covering the sensing layer 9. The diffusion barrier of sensors F to J was provided as a polymer layer made of MPC-like layer 11 in figure 1. As can be seen, the sensor currents are only slightly lower for sensors F to J than for sensors A to E. Hence, the diffusion barrier provided by the MPC polymer layer 11 hinders diffusion of analyte molecules only to a very small extent. As the sensor currents of sensors F to J are about 20% lower than sensor currents of sensors A to E, it can be concluded that the diffusion barrier of sensors F to J leads to analyte concentrations on the upper surface of the sensing layer 9 which are only about 20% lower than in the glucose solution surrounding the sensors.

As in the embodiment described previously with reference to figure 1 the sensing layers 9 of sensors A to J comprise cross-linked enzymes, i.e. dextranized glucose oxidase which can be purchased by Roche Diagnostics, Penzberg, Germany, ident-No. 14859389001. The dextranized glucose oxidase has been dissolved in phosphate-buffered solution and mixed into a paste comprising carbon particles, carbon nanotubes and polymeric binder. The sensing layer 9 was dispensed on the contact pad 7 of the working electrode 3 on the sensor substrate 5 with a spot size of about 0.05 mm^2 to 0.1 mm^2, e.g. a circular spot of 300 µm diameter. The thickness of the sensing layer 9 was 20 µm. An Ag/AgCl reference electrode 4 of identical size has also been provided. The counter electrode 2 was of rectangular shape (400 µm by 900 µm) with a 20 µm thick layer of carbon paste containing carbon nanotubes.

It is seen in figure 4 that the sensor current is barely affected by the presence of the membrane made from MPC.

It can be concluded from this finding that, by the particular choice of the zwitterionic membrane structure, a coating which is highly permeable for solvated glucose has been found. For the construction of a sensor 1, where diffusion-limitation occurs in the sensing layer 9 (see figure 1), such a high permeability of the membrane is important. Vice versa, the diffusion of analyte through the diffusion barrier provided by the MPC-layer 11, should be hindered as little as possible, ideally the analyte concentration (i.e. signal) at the sensing layer 9 with the coating should be not less than half of the value obtained without the coating.

It should be noted that the optional enzyme-free layer 10 should also have little hindrance to analyte diffusion, therefore its layer thickness should be much thinner than that of the sensing layer 9.

As stated before, mixing of a hydrophilized cross-linked enzyme can yield a very stable function over extended periods of time, since wet-up of the sensing layer is fast and the enzyme distribution stays constant. This is reflected by drift values obtained in measuring the above sensors over 6

days in aqueous glucose solution. For the uncoated sensors, drift ranges from -0.62% per day to 0.78% per day, while the coated ones cover a range from -0.5% to 1.5% per day. These small drift values have been measured at 37 °C.

The particular advantage of measurement stability, i.e. low signal drift, is not limited to a sensor 1 with an enzyme in the sensing layer 9 which uses oxygen as a co-substrate. In fact, the same benefit of cross-linking can be obtained by using a cross-linked dehydrogenase which does not need oxygen as a co-substrate in the catalytic reaction. For example, a dextranised glucose dehydrogenase or a pegylated dehydrogenase (PEG: polyethylene glycol) can be brought into the sensing layer 9.

In the sensor 1 shown in figure 1 the sensing layer 9 is arranged on the contact pad 7. Further, the sensing layer 9 has a lower surface facing the contact pad 7 and an upper surface facing away from the contact pad 7, or more generally the sensing layer 9 has having a lower surface facing the support member 5 and an upper surface facing away from the support member 5 toward the analyte-containing body fluid. Correspondingly the layers 12, 14 are arranged the contact pads 6, 8. Figure 5 shows an amended embodiment of the sensor 1 of figure 1. The embodiment of figure 5 corresponds to the embodiment of figure 1, with the difference that the electrodes with the contact pads 6, 7, 8 being placed on the side of the water-permeable layers 9, 12 and 14, in contrast to figure 1. It is also possible to place a contact pad 6, 7, 8 on two sides of the respective layer 9, 12, 14, as illustrated for the contact pad 6 of the water-permeable layer 12 of counter electrode 2. This contact pad 6 can also be formed so that it encloses the layer 12 from all sides. In all cases where the contact pad 6, 7, 8 sits on the side of the permeable layer 9, 12, 14, the surface of the layer 9, 12, 14 facing away from the analyte-containing body fluid is directly in contact with the support member 5.

RDG 303/0A/WO

List of reference numbers

1	sensor
2	counter electrode
3	working electrode
4	reference electrode
5	support member
6	contact pad of 2
7	contact pad of 3
8	contact pad of 4
9	sensing layer
10	enzyme-free layer
11	layer (dialysis layer, polymer layer, MPC-layer)
12	water-permeable layer of 2
13	porosity enhancing particles (porous particles)
14	water-permeable layer of 4

Patent claims

1. An amperometric sensor (1) configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said sensor (1) comprising

a counter electrode (2) and

a working electrode (3),

said working electrode (3) comprising a sensing layer (9) which is permeable for water and arranged on a support member (5) adjacent to a contact pad (7), said sensing layer (9) comprising an immobilized enzyme capable of acting catalytically in the presence of the analyte to cause an electrical signal,

the sensing layer (9) having an upper surface facing the body fluid and a lower surface facing away from the body fluid,

characterized in that

the immobilized enzyme is distributed in the sensing layer (9) in such a way that the enzyme concentration in the middle between its upper and lower surface is at least as high as on the upper surface of the sensing layer (9).

2. Sensor (1) according to claim 1, wherein the contact pad (7) of the working electrode (3) is an electrically conductive film.

3. Sensor (1) according to claim 1 or 2, wherein the contact pad (7) of the working electrode (3) is a metallic film or an electrically conductive polymer film.

4. Sensor (1) according to any of the preceding claims, wherein the working electrode (3) is arranged on a support member (5), especially a support member (5) made of a plastic material.

5. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) has a thickness of at least 5 µm, especially at least 10 µm.

6. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) contains carbon particles and a polymeric binder.

7. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) contains porous particles (13), especially silica and/or carbon nanotubes.

8. Sensor (1) according to claim 7, wherein the porous particles (13) measure at least 1 µm, especially at least 5 µm, on average.

9. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) is covered by a diffusion barrier which hinders diffusion of analyte molecules to such an extent that after implantation into the living body of a human or an animal the analyte concentration at the upper surface of the sensing layer (9) is at most ten times lower than in the body fluid surrounding the implanted sensor (1), especially at most five times lower.

10. Sensor (1) according to claim 9, wherein the diffusion barrier comprises an electrically conductive enzyme-free layer (10) comprising carbon particles and a polymeric binder.

11. Sensor (1) according to claim 9 or 10, wherein the diffusion barrier comprises a dialysis layer (11).

12. Sensor (1) according to any of claims 9 to 11, wherein the diffusion barrier comprises a polymer layer (11) made of a polymer having a zwitterionic structure.

13. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) is flat.

14. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) has lateral surfaces which are impervious for the body fluid.

15. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) is electrically conductive.

16. Sensor (1) according to claim 15, wherein the sensing layer (9) has an electrical conductivity of at least 1 Ω^{-1} cm^{-1}.

17. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) is adapted and arranged in such a way that in operation after implantation the analyte concentration in the sensing layer (9) is highest at the upper surface, decreases with increasing distance from the upper surface, and is zero at the lower surface which is the furthest point from the analyte-containing body fluid.

18. Sensor (1) according to any of the preceding claims, wherein the enzyme is an oxidase, especially a glucose oxidase.

19. Sensor (1) according to any of the preceding claims, where the enzyme is a dehydrogenase, especially glucose dehydrogenase.

20. Sensor (1) according to any of the preceding claims, wherein the enzyme is a cross-linked enzyme.

21. Sensor (1) according to claim 20, wherein the cross-linked enzyme has an average chain length of three to ten, especially four to eight, enzyme molecules.

22. Sensor (1) according to any of the preceding claims, wherein the enzyme is distributed equally throughout the sensing layer (9).

23. Sensor (1) according to any of the preceding claims, wherein the sensing layer (9) has an effective diffusion coefficient D_{eff}, which characterizes the diffusion of the analyte in the sensing layer (9) and is 10-times to 1000-times lower than the diffusion coefficient D of the analyte in water.

24. Sensor (1) according to claim 23, wherein the sensing layer (9) has an enzyme loading parameter α which is between 10 to 200 times smaller than the effective diffusion coefficient D_{eff}.

25. Sensor (1) according to any of the preceding claims, wherein said sensing layer (9) is arranged on the contact pad (7) and the sensing layer (9) has a lower surface facing the contact pad (7) and an upper surface facing away from the contact pad (7).

26. Method for manufacturing an amperometric sensor (1) configured for implantation into the living body of a human or animal to measure the concentration of an analyte in a body fluid, said method comprising the following steps:
- mixing carbon particles, enzyme and a polymeric binder to create a paste,
- applying that paste adjacent to a contact pad (7) onto a support member (5),
- hardening that paste into a porous sensing layer (9).

27. Method according to claim 26, wherein cross-linked enzyme is mixed with carbon particles and a polymeric binder.

28. Method according to claim 26 or 27, wherein the sensing layer (9) is manufactured such that it has an effective diffusion coefficient D_{eff}, which characterizes the diffusion of the analyte in the sensing layer (9) and is 10-times to 1000-times lower than the diffusion coefficient D of the analyte in water.

29. Method according to any of claims 26 to 28, wherein the sensing layer (9) is manufactured such that it is electrically conductive.

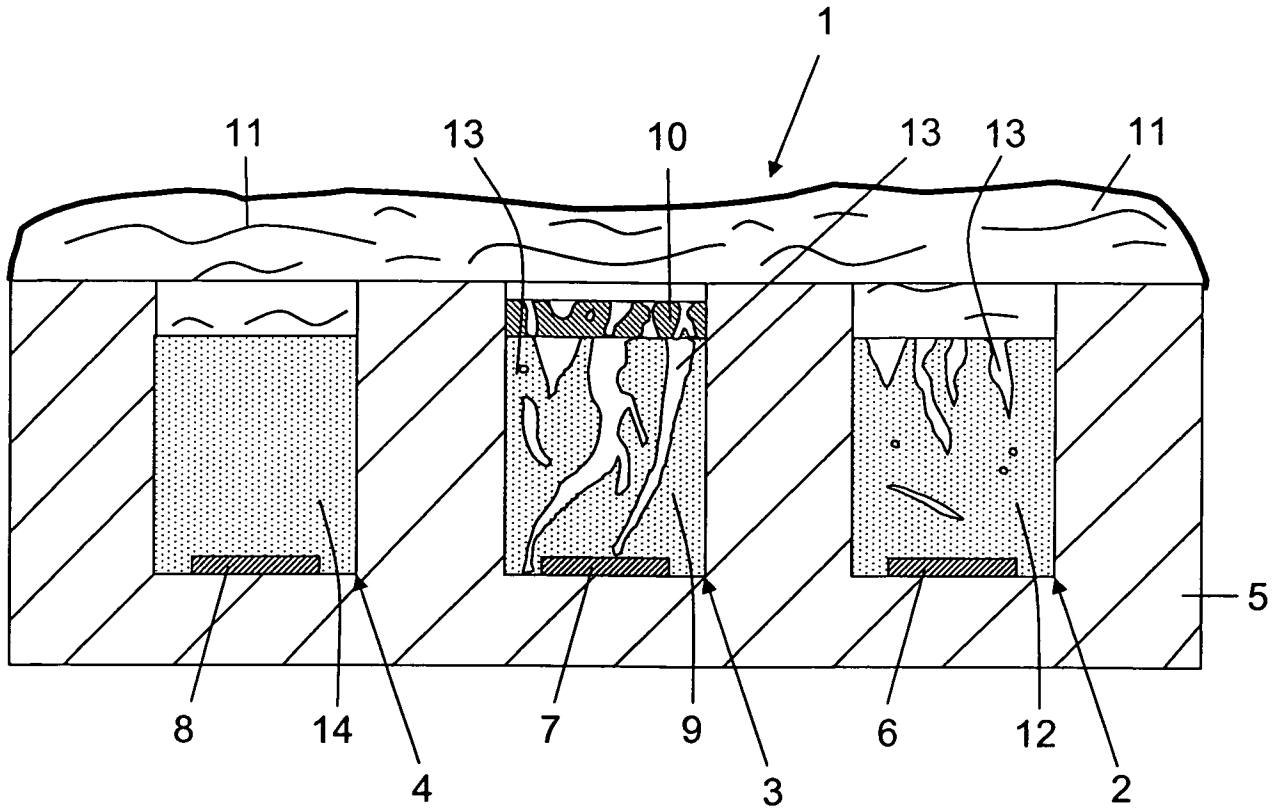
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Fig. 1

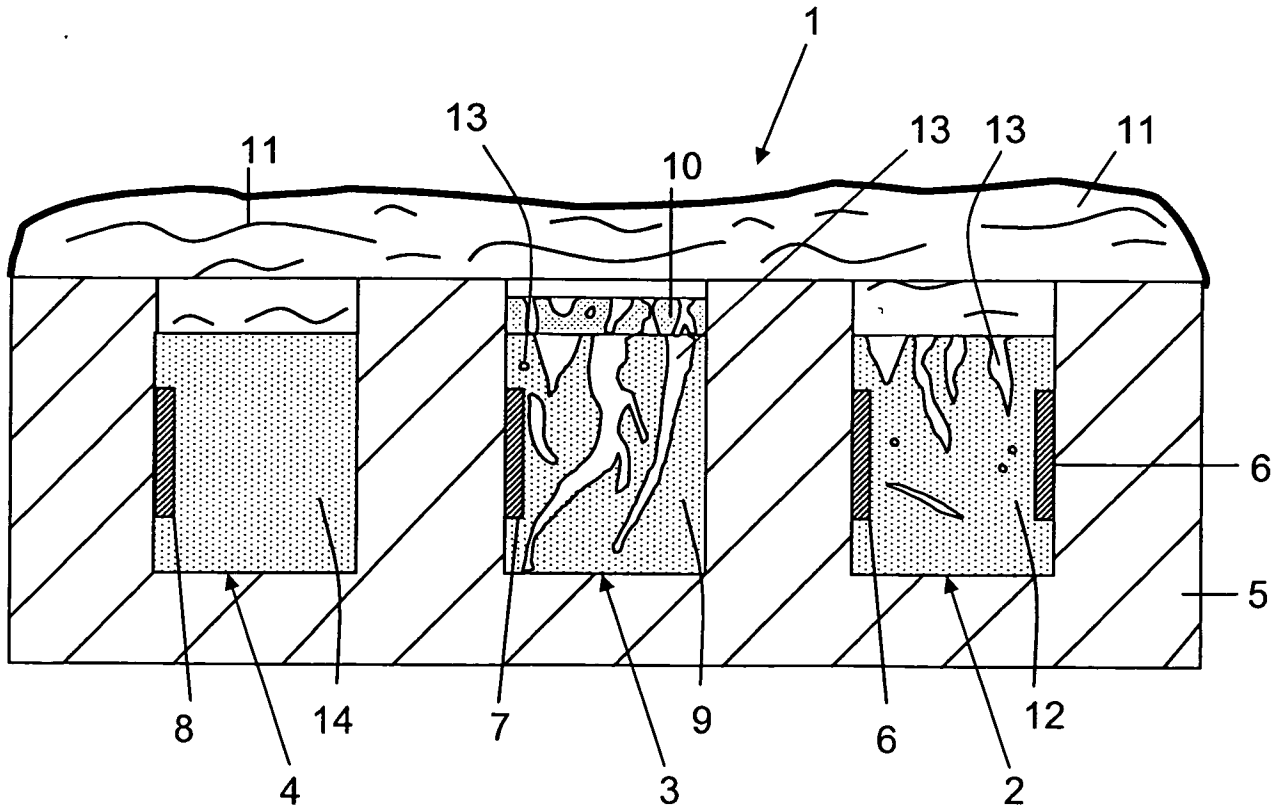
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Fig. 5

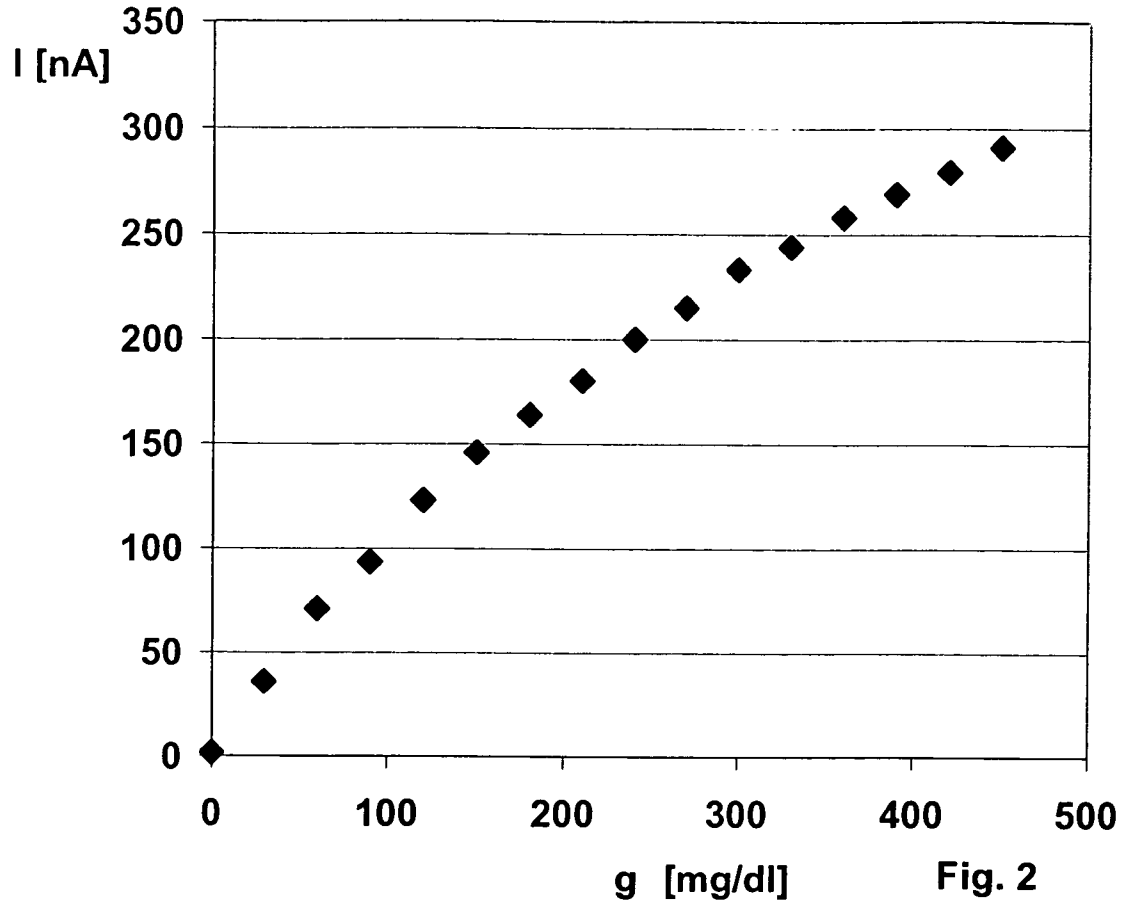
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Fig. 2

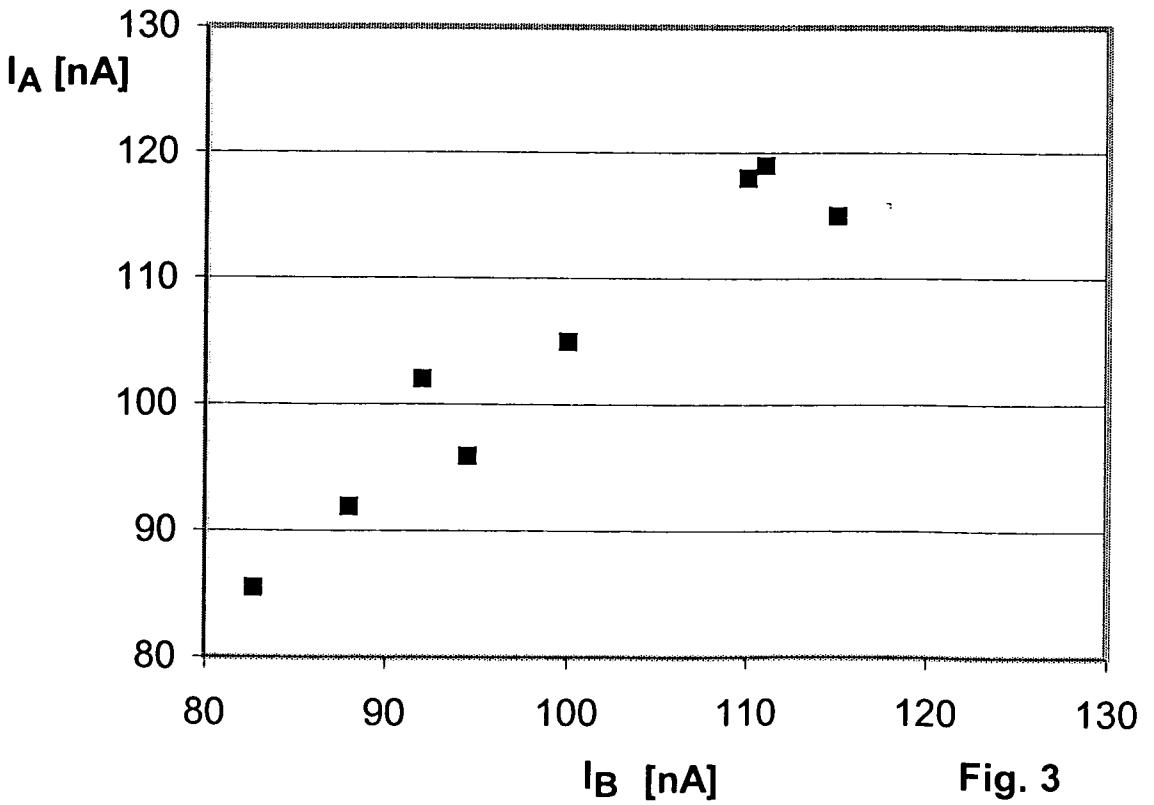
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Fig. 3

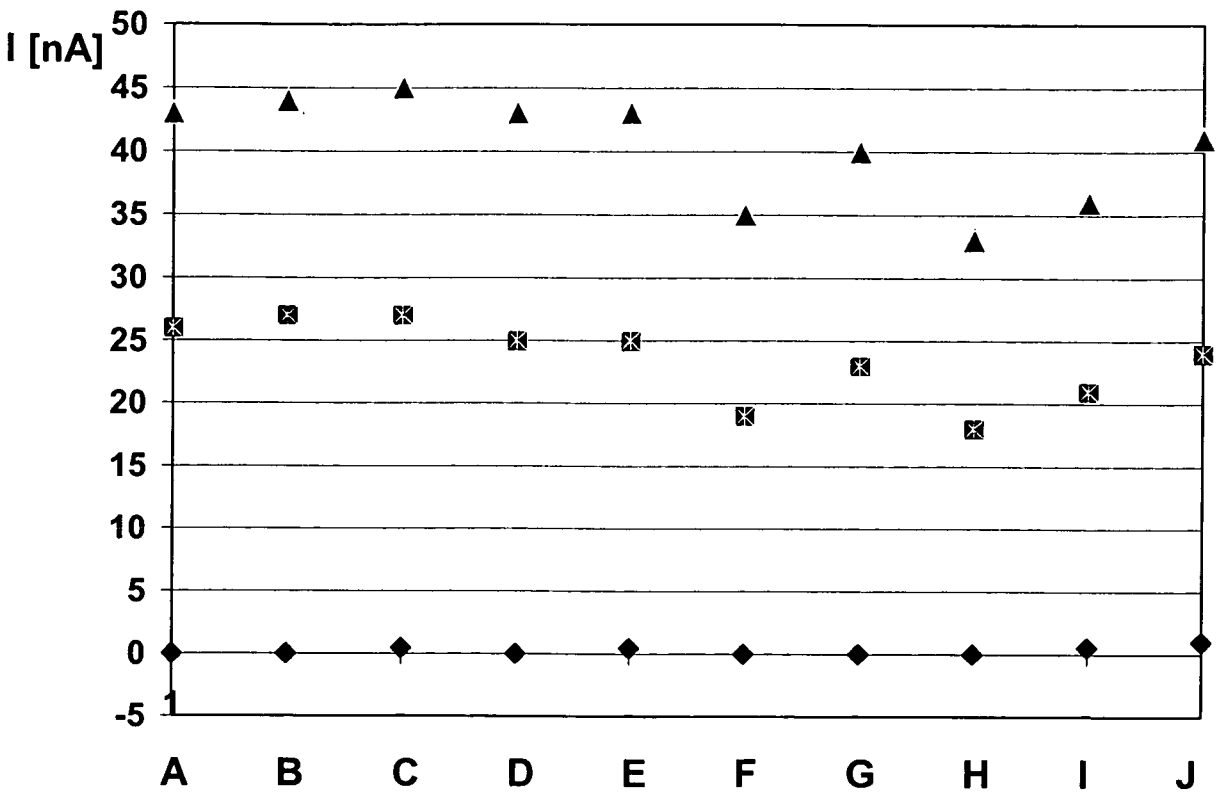

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/004606

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/487 G01N27/49

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)
A61B G01N C12Q

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MANG A ET AL: "Biocompatibility of an electrochemical sensor for continuous glucose monitoring in subcutaneous tissue." DIABETES TECHNOLOGY & THERAPEUTICS FEB 2005, vol. 7, no. 1, February 2005 (2005-02), pages 163-173, XP002446481 ISSN: 1520-9156 page 164, right-hand column, paragraph 3 - page 165, left-hand column, paragraph 1 figure 1 ----- -/--	1-29

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2007	22/08/2007

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Baranski, Jörg

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/004606

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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