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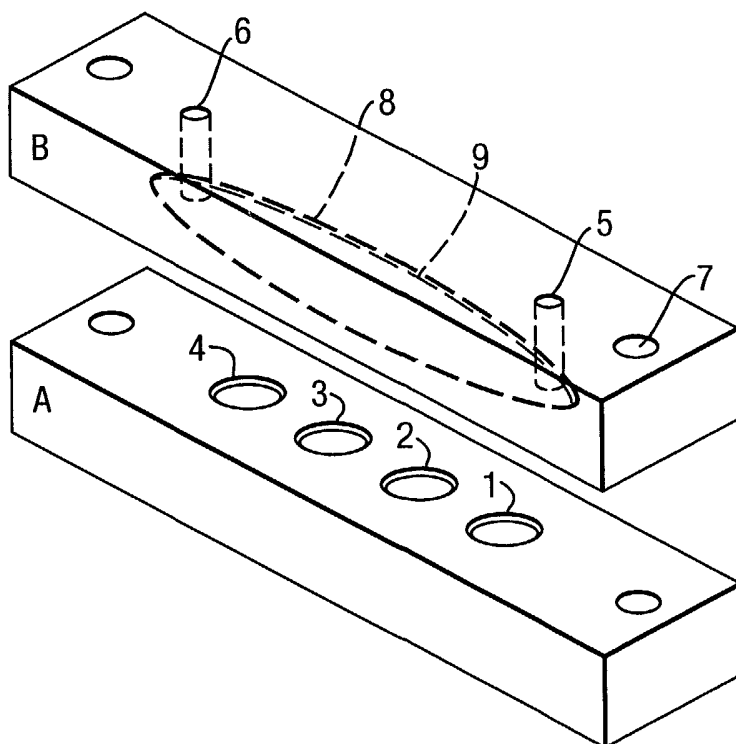
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(54) Title: AMPEROMETRIC SENSOR



(57) Abstract: An amperometric sensor suitable for determining the concentration of analyte in a sample, said sensor comprising an oxidase enzyme and a substituted 1,4-benzoquinone compound which, in oxidized form, functions as a mediator specific to reduced enzyme and which has a lower oxidation potential than unsubstituted 1,4-benzoquinone.



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AMPEROMETRIC SENSOR

In general terms the present invention relates to the determination of the concentration of an analyte in a sample. More specifically, the invention relates to an amperometric sensor, to its use, to cartridges for the sensor and to redox mediator compounds for use in the sensor.

A number of electrochemical sensors (or biosensors) have been proposed previously. For example, US 5,288,636 describes a sensor useful for determining glucose concentration in a sample and relies on the reaction between the enzyme glucose oxidase and glucose with the mediator potassium ferricyanide to produce a ferrocyanide which is then electro-oxidised to produce a measurable current that is representative of the concentration of glucose present.

The reactions involved can be summarised as follows:

1. $GOD_{OX} + \text{glucose} \rightarrow GOD_{RED} + \text{gluconolactone}$
2. $GOD_{RED} + M_{OX} \rightarrow GOD_{OX} + M_{RED}$
3. $M_{RED} \rightarrow M_{OX} + e^- [\text{Signal}]$

GOD_{OX} – oxidised form of glucose oxidase

GOD_{RED} – reduced form of glucose oxidase

M_{OX} – oxidised form of mediator (ferricyanide)

M_{RED} – reduced form of mediator (ferrocyanide)

In step 1 the enzyme oxidises the glucose and is itself reduced. In step 2 the reduced form of the enzyme reacts with the oxidised form of the mediator to produce the reduced form of the mediator and to regenerate the oxidized form of the enzyme. In step 3 the oxidised form of the mediator is regenerated by electro-oxidation. A measurable current/signal is generated. Thus, this type of sensor depends on reaction between the mediator and enzyme.

The use of a quinone-hydroquinone mediator system with glucose oxidase is known (Stoytcheva et al, *Analytica Chimica Acta*, 315 (1995) pp 101-107). However, unsubstituted benzoquinone has the disadvantage of having a relatively high oxidation potential of approximately +400 mV (versus SCE).

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US 4,711,245 also describes a sensor for determining glucose concentration. The sensor relies on a reaction involving glucose, the enzyme glucose oxidase and the oxidised form of a substituted ferrocene. The ferrocene is reduced and then reoxidised to produce an easily measurable current.

5 There are various advantages associated with mediated sensors. Firstly, the kinetics for the oxidation of reduced enzyme can be faster with the mediator than with the natural electron acceptor oxygen. This can result in the sensor response being independent of oxygen tension. Secondly, in known sensors a potential is applied between electrodes in order to oxidize the reduced form of the mediator. The
10 potentials sufficient to achieve this can be lower than for known sensors based on oxidation of hydrogen peroxide. This can result in a reduced oxidation of interferants present in the system, for example, ascorbate, urate and paracetamol.

The solubility of the mediator is an important factor in obtaining a measurable sensor response. The use of mediated sensors in flow systems can be
15 limited by the solubility of the mediator. A readily soluble mediator can be lost rapidly to the carrier solution, resulting in severely reduced operating lifetime of the sensor. Alternatively, where the sensor is used in static solution and possibly with minimal sample volume, such as an electrochemical blood glucose stick, the use of a mediated sensor is enhanced by the solubility of the mediator. A poorly soluble
20 mediator will limit the mediated enzyme reaction, resulting in poor sensitivity of the sensor.

The present invention provides mediators suitable for use in flowing streams by use of a sensor reliant on relatively insoluble mediator. The present invention also provides mediators suitable for use in static solution by use of a sensor reliant on
25 relatively soluble mediator.

Accordingly, the present invention provides an amperometric sensor suitable for determining the concentration of analyte in a sample, said sensor comprising an oxidase enzyme and a substituted 1,4-benzoquinone compound which, in oxidized form, functions as a mediator selective for reduced enzyme and which has an
30 oxidation potential lower than that of unsubstituted 1,4-benzoquinone.

The enzyme is an oxidase type enzyme. For example, in a sensor for

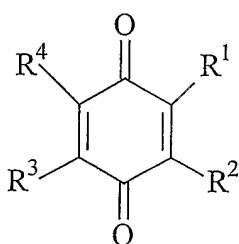
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determining the concentration of glucose in a sample, the enzyme may be glucose oxidase. The reaction between the enzyme and the analyte yields reduced glucose oxidase, and the concentration of the reduced glucose oxidase can be determined by using the sensor response and correlating this to a corresponding glucose
5 concentration.

Other analytes which may be determined using the sensor of the present invention include cholesterol, pyruvate, bilirubin, alcohol, lactate, sarcosine, glycerol, creatinine, triglycerides and cholesterol; USP 5,288,636 gives details of some of the relevant enzymes. These analytes may be measured if one or more
10 suitable enzyme(s) and mediator are included in the sensor. The sensors are constructed so that the final enzyme reaction results in the formation of reduced enzyme, which can be detected by the substituted benzoquinone mediator.

Herein the term "mediator" as used herein, means a compound which is capable of undergoing an electrochemical, reversible oxidation-reduction reaction.

The mediator used in the present invention is a substituted 1,4-benzoquinone
15 compound which in oxidized form is selective for the redox site of reduced enzyme, i.e. which is reduced on reaction with reduced enzyme. The mediator has satisfactory solubility in water and common organic solvents, and has a lower redox potential than the corresponding unsubstituted benzoquinone. Examples of suitable
20 compounds include those of the following formulae (I):



(I)

wherein R^1 , R^2 , R^3 and R^4 are the same or different and at least one of R^1 , R^2 , R^3 and R^4 is an alkyl group preferably C_{1-3} alkyl or a phenyl group. Alternatively the groups R^1 , R^2 , R^3 and R^4 may be chosen from an alkoxy group or a hydroxyalkyl group,
25 preferably C_{1-3} alkoxy or hydroxy C_{1-3} alkyl. Preferably the substituents are chosen from methyl, methoxy and hydroxymethyl groups.

Suitable 1,4-benzoquinone mediators for use in the sensor of the invention include:

- methyl-1,4-benzoquinone
ethyl-1,4-benzoquinone
propyl-1,4-benzoquinone
2,5-dimethyl-1,4-benzoquinone
5 2,6-dimethyl-1,4-benzoquinone
trimethyl-1,4-benzoquinone
tetramethyl-1,4-benzoquinone
methoxy-1,4-benzoquinone
ethoxy-1,4-benzoquinone
10 propoxy-1,4-benzoquinone
2,5-dimethoxy-1,4-benzoquinone
2,6-dimethoxy-1,4-benzoquinone
trimethoxy-1,4-benzoquinone
tetramethoxy-1,4-benzoquinone
15 hydroxymethyl-1,4-benzoquinone
hydroxyethyl-1,4-benzoquinone
hydroxypropyl-1,4-benzoquinone
2,5-dihydroxymethyl-1,4-benzoquinone
2,6-dihydroxymethyl-1,4-benzoquinone
20 tri(hydroxymethyl)-1,4-benzoquinone
tetra(hydroxymethyl)-1,4-benzoquinone
phenyl-1,4-benzoquinone
2,3-dimethoxy-5-methyl-1,4-benzoquinone, and
2-hydroxymethyl-6-methoxy-1,4-benzoquinone.

25 An advantage of the substituted benzoquinones as defined herein is a reduction in oxidation potential compared to unsubstituted benzoquinone which has an oxidation potential of approximately + 400 mV (versus SCE). It has been shown that addition of methyl groups to 1,4-benzoquinone results in a negative shift in redox potential of approximately 55 mV per methyl group, due to the electron-
30 donating nature of methyl groups (Driebergen et al., *Analytica Chimica Acta*, 233 (1990) pp 251-268). This enables measurements to be performed at lower oxidation potential and reduces the oxidation of interferent species present in samples such as

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ascorbate, urate and paracetamol. Examples of substituted 1,4-benzoquinones with lower oxidation potentials compared to 1,4-benzoquinone are shown in Table 1 below:

Compound	Solubility (mg/L)	E _{ox} (mV)
1,4-benzoquinone	1.11 E+04 (SRC)	278
2,6-dimethyl-1,4-benzoquinone	7.88 E+03 (SRC)	137
Tetramethyl-1,4-benzoquinone	4.43 E+02(SRC)	-37
Phenyl-1,4-benzoquinone	1.14 E+03 (SRC)	229
2,3-dimethoxy-5-methyl-1,4-benzoquinone	1.11 E+04 (SRC)	156
2,6-dimethoxy-1,4-benzoquinone	7.06 E+04 (SRC)	166
2-hydroxymethyl-6-methoxy-1,4-benzoquinone	2.85 E+05 (C)	158

SRC = values obtained from SRC PhysProp Database at

<http://esc.syrres.com/interkow/>

E_{ox} = potential of oxidation peak obtained at a glassy carbon electrode, using pH 6.95 bis-tris buffer and a Ag/AgCl/sat. KCl reference electrode.

C = calculated using KowWin from SRC PhysProp Database at

<http://esc.syrres.com>

5

An additional advantage of use of the substituted benzoquinones is in the ability of these mediator compounds to oxidize reduced enzyme in place of the physiological electron acceptor, oxygen. This results in a sensor response that is independent of the oxygen tension of the sample. This is an advantage compared to sensors based on the measurement of hydrogen peroxide production or oxygen depletion caused by the reaction between enzyme and substrate.

Suitable benzoquinones for use in the sensor of the invention may be selected on the basis of their solubility compared to benzoquinone. The solubility of the substituted benzoquinones can be reduced by substitution of groups such as alkyl or aryl groups onto the ring. Solubility of the compound of formula (I) is an important

15

factor in the proper functioning of the sensor in a flowing stream. Low solubility in water and aqueous phases is helpful in providing stability and conveniently, for use in a flowing stream, the compound of formula (I) should have a solubility of from 400 mg/L to 10,000 mg/L in pure water.

5 Sensors of the invention for use in static solution will advantageously comprise substituted benzoquinones which have higher solubility compared to unsubstituted benzoquinone. The solubility of the substituted benzoquinones can be increased by substitution of groups such as hydroxy, alkoxy or hydroxyalkyl onto the ring. Relatively high solubility in water and aqueous phases is helpful in providing
10 enhanced sensitivity of the sensor response in static solution. Conveniently in this embodiment, the compound of formula (I) will have a solubility of at least 10,000 mg/L in pure water.

Solubility in common organic solvents is desirable to facilitate fabrication of the sensors of the present invention, and conveniently the compound of formula (I)
15 will have a solubility of at least 20,000 mg/L and preferably higher, in at least one of methanol, ethanol, propanol, other lower alkanols, chloroform, dichloromethane or other chlorinated alkanes and acetone and other low molecular weight solvents.

Preferably the mediator is specific to reduced enzymes, i.e. under the conditions of the analysis, the mediator only accepts electrons from the redox site of
20 a reduced enzyme. In practice it is likely that this will be the case when operating at the preferred potential (see below). However, specificity is not essential and the system may be operated satisfactorily provided that the mediator is selective for reduced enzyme, i.e. under the conditions of the analysis the mediator tends to accept
25 electrons from the redox site of the reduced enzyme in preference to any other electron donor available to the mediator.

In one embodiment of the invention, groups R^1 , R^2 , R^3 and R^4 may be selected from any alkyl group, preferably C_{1-3} alkyl. The alkyl group includes methyl, ethyl and propyl and is preferably methyl. Another suitable configuration has R^1 , R^2 and R^3 all hydrogen atoms and group R^4 a phenyl group. If there is more
30 than one alkyl group or a phenyl group, these substituents render the substituted benzoquinone relatively insoluble which provides a mediator of particular advantage for use in a flowing stream. It is preferred in that embodiment that the substituted

benzoquinones used have a solubility of not more than 10,000 mgL⁻¹ in water at room temperature (25°C), and not less than 1000 mgL⁻¹. As specific examples of useful compounds there may be mentioned 2,6-dimethyl-1,4-benzoquinone, which has a solubility of 7888 mgL⁻¹ in water at 25°C, tetramethyl-1,4-benzoquinone, which has a solubility of 443 mgL⁻¹ in water at 25°C, phenyl-1,4-benzoquinone, which has a solubility of 1135 mgL⁻¹ in water at 25°C, and 2,5-dimethyl-1,4-benzoquinone, which has a solubility of 7010 mgL⁻¹ in water at 25°C, which may be compared to the parent compound 1,4-benzoquinone which has a solubility of 11,000 mgL⁻¹ in water at 18°C.

10 An alkyl group or groups may be substituted by one or more substituents provided that these do not have a detrimental effect on the activity of the mediator compounds.

In another embodiment of the invention, groups R¹, R², R³ or R⁴ may be an alkoxy group or a hydroxyalkyl group, preferably C₁₋₃ alkoxy or hydroxy C₁₋₃ alkyl. The alkoxy group includes methoxy, ethoxy and propoxy and is preferably methoxy. 15 The hydroxyalkyl group includes hydroxymethyl, hydroxyethyl and hydroxypropyl and is preferably hydroxymethyl. If there is more than one alkoxy group or hydroxyalkyl group, these substituents render the substituted benzoquinone relatively soluble which provides mediator of particular advantage for use in static solution. It is preferred that in this aspect of the invention, that the substituted benzoquinones used have a solubility of at least 10,000 mgL⁻¹ in water at room temperature (25°C). As specific examples of useful compounds there may be mentioned 2,6-dimethoxy-1,4-benzoquinone which has a solubility of 70,600 mgL⁻¹ in water at 25°C, 2,3-dimethoxy-5-methyl-1,4-benzoquinone which has a solubility of 11,000 mgL⁻¹ in 20 water at 25°C and 2-hydroxymethyl-6-methoxy-1,4-benzoquinone.

Some of the compounds useful as mediators are known and are commercially available. Alternatively, they may be made by the application or adaptation of known techniques.

The mediator compounds disclosed herein are useful in a variety of 30 amperometric sensor devices and electrode configurations. The sensors may be based on a 2 or 3 electrode system and may be of the disposable (single use) or reusable/semi-disposable type. The sensors may be used either in a flowing stream of

solution or in static solution, depending on the relative solubility of the mediator used. In its simplest form the sensor comprises two electrodes (working and counter) which in use are contacted with the sample being analysed. One electrode, the working electrode, is coated with the mediator compound. Mediator may be applied to the electrode by deposition from a solution of the mediator in a readily evaporable organic liquid. For mediator that is fairly soluble in aqueous solution, it may also be applied to the electrode by deposition from an aqueous solution of the mediator.

When the sensor is being used to determine the concentration of an analyte such as glucose the mediator is coated with a suitable enzyme. The enzyme can be immobilised on the surface of the mediator by conventional techniques such as by use of a self-sustaining gel layer and/or by use of a retention layer, which is permeable to the analyte. US 4,711,245 describes in greater detail ways in which the mediator and, when used, the enzyme may be fixed on the working electrode.

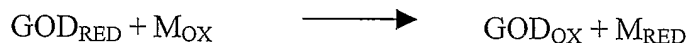
The electrode substrate is chosen from conventional materials such as carbon pellets, carbon inks, metallized carbon and metals (such as platinum or palladium), carbon rods, pencil leads and carbon rods loaded with metal powder. Conventional electrode configurations which may be used include those disclosed in US 4,711,245, US 5,200,051 and US 5,288,636, incorporated herein by reference. The basic chemical and electrochemical transformations associated with the present invention are shown below with reference to the glucose/glucose oxidase system. Prior to introduction of the sample to be analysed a potential of about +200mV (versus Ag/AgCl) is applied to the sensor electrode. This potential is sufficient to cause oxidation of the mediator at the working electrode, i.e. conversion of hydroquinone to the corresponding benzoquinone. When the electrodes are contacted with the sample to be analysed, the enzyme at the working electrode acts on the glucose resulting in the formation of the reduced form of the enzyme. The reaction proceeds as in the reaction scheme below:



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The reduced form of the enzyme reduces the oxidized form of the mediator as follows:

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Then, under the applied potential, the reduced form of the mediator at the
5 working electrode is converted to the oxidized form and a diffusion limited current
generated. This current can be measured and correlated to the concentration of
analyte in the sample.

At the electrode potential involved (+200 mV) there may be some oxidation
of interferants. Typically in blood and plasma samples these are ascorbate, urate and
10 paracetamol. A diffusion limiting layer may be applied to the working electrode to
extend the sensor to measurement of higher analyte concentrations. This layer may
also reduce the diffusion of some interferents to the electrode by acting as a
diffusion-limiting barrier. This layer can also act as an electrostatic barrier to
diffusion of interferents if it contains negatively charged groups. Examples of
15 materials for use as the diffusion-limiting layer include NafionTM and cellulose
acetate. An effective outer membrane results in a sensor response which is an
accurate reflection of the reduced enzyme concentration in the sample. The reduced
enzyme concentration may be correlated to analyte concentration.

It is envisaged that the sensors of the invention will find most practical utility
20 in the measurement of glucose in blood samples, although they may be also used for
other medical and non-medical applications, for example in the food industry.

Brief description of the accompanying drawings:

Figure 1:

A sketch of an embodiment of the amperometric sensor of the invention in
25 the form of a flow cell. The cell consists of a lower part (A) containing the sensors
and an upper part (B) containing the inlet and outlet for fluid flow. Each sensor is
surrounded by a recess of 0.5 mm width and 0.1 mm depth. The top of the sensor
surface is level with the bottom of the recess. The upper part (B) contains a rubber O-
ring seal and is clamped in place over the sensors. The parts shown are: sensor 1 (1),
30 sensor 2 (2), Ag/AgCl reference electrode (3), sensor 3 (4), flow inlet (5), flow outlet
(6), hole for screw (7), O-ring (8) and 0.2 mm recess over sensors and between inlet
and outlet (9).

Figure 2A:

Real time calibration traces obtained by flow injection from three 2,6-dimethyl-1,4-benzoquinone mediated glucose sensors showing the responses to injections of glucose solutions containing 5, 10, 20, 40, 62.5 and 125 mM glucose.

5 The carrier used was bis-tris saline buffer, pH 6.95.

Figure 2B:

The calibration plots for the three 2,6-dimethyl-1,4-benzoquinone mediated glucose sensor responses shown in Figure 2B.

10

Figure 3A:

Real time calibration traces obtained by flow injection from three phenyl-1,4-benzoquinone mediated glucose sensors showing the responses to injections of glucose solutions containing 2.5, 5, 20, 40 and 62.5 mM glucose. The carrier used

15

Figure 3B:

The calibration plots for the three phenyl-1,4-benzoquinone mediated glucose sensor responses shown in Figure 3B.

20

Figures 4A and 4B show the current response and the corresponding calibration plot in the response of 2,3-dimethoxy-5-methyl-1,4-benzoquinone/sarcosine oxidase to sarcosine.

Figures 5A and 5B show the current response and the corresponding calibration plot in the response of 2,6-dimethyl-1,4-benzoquinone/sarcosine oxidase to sarcosine.

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Figures 6A and 6B show the current response and the corresponding calibration plot in the response of 2,5-dimethyl-1,4-benzoquinone/sarcosine oxidase to sarcosine.

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Figure 7A and 7B show the current response and the corresponding calibration plot in the response of 2,5-dimethyl-1,4-benzoquinone/glucose oxidase to glucose.

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Figures 8A and 8B show the current response and the corresponding calibration plot in the response of 2,3-dimethoxy-5-methyl-1,4-benzoquinone/glucose oxidase to glucose.

Figures 9A and 9B show the current response and the corresponding calibration plot in the response of 2-hydroxymethyl-6-methoxy-1,4-benzoquinone/glucose oxidase to glucose.

EXAMPLES

10 Example 1 Electrode construction

The amperometric flow cell was supplied by Drew Scientific, Cumbria, U.K. Figure 1 shows a sketch of the flow cell, including the sensors. The cell was made of either PVC or nylon. Carbon pellets (2mm diameter and 4mm length) were press fitted into the holes in the lower part (A) so that the front face was flush with the bottom of the recess. Electrical contact was made to the back face of the pellets. One of the carbon pellets was modified with Ag/AgCl paste to act as both a pseudo-reference electrode and counter electrode.

20 Example 2 2,6-dimethyl-1,4-benzoquinone mediated glucose sensor

Unmodified carbon pellets, inserted into the bottom part of the flow cell as described in Example 1, were prepared as glucose sensors by successively depositing a mediator layer, an enzyme layer and an outer diffusion limitation layer. 2 - 4 μL of 0.5 M 2,6-dimethyl-1,4-benzoquinone solution in acetone was deposited into the recess surrounding each pellet and allowed to dry for 3 minutes. A solution containing a mixture of glucose oxidase GOX (2 - 4 U/ μL), PVA (0.09%) and Nafion (0.31%) was prepared and a total of 4 μL of the solution was deposited on top of the benzoquinone layer of each sensor to form the enzyme sensing layer. The sensors were air dried for 20 minutes before a total of 3 μL of 2.5% Nafion solution was deposited onto each sensor. The sensors were air dried for at least 4hr before use.

Example 3 Use of 2,6-dimethyl-1,4-benzoquinone glucose sensor

Flow injection chronoamperometry was used to show the activity of these
5 sensors to glucose. A two electrode system was used, with the three sensors of
Example 2 as the working electrodes, and the pellet modified with silver/silver
chloride acting as the reference and counter electrode. An AutoLab (Eco Chemie
B.V.) electrochemical system was used for the measurements. The carrier buffer was
pH 6.95 bis-tris buffer (40 mM), containing 142 mM NaCl, 0.8 mM Na₂EDTA and
10 4.2 mM KCl. The working electrodes were poised at a potential of +200 mV versus
the silver/silver chloride reference electrode. Figure 2A shows anodic current peaks
corresponding to injections of glucose. Calibration plots resulting from the
amperometry data in Figure 2A are shown in Figure 2B. The linear range of response
is from 0 to 60 mM glucose.

15

Example 4 Phenyl-1,4-benzoquinone mediated glucose sensor

Unmodified carbon pellets, inserted into the bottom part of the flow cell as
described in Example 1, were prepared as glucose sensors in an identical manner to
20 the sensors in Example 2, with the exception of the use of 0.5 M phenyl-1,4-
benzoquinone solution in place of 2,6-dimethyl-1,4-benzoquinone solution.

Example 5 Use of phenyl-1,4-benzoquinone glucose sensor

25 Flow injection chronoamperometry was used to demonstrate the response of
the sensors in Example 4 to glucose, using the same experimental methodology given
in Example 3. Figure 3A shows anodic current peaks corresponding to injections of
glucose. Calibration plots resulting from the amperometry data in Figure 3A are
shown in Figure 3B. The linear range of response is from 0 to 60 mM glucose.

Example 6

Chronoamperometry was used to show the mediator ability of some compounds in solution to sarcosine oxidase. The change in current at a glassy carbon electrode was observed on consecutive addition of aliquots of 1 M sarcosine stock solution to buffer (pH 7.4) containing 100 units/ml sarcosine oxidase and either 0.2 M 2,6-dimethyl-1,4-benzoquinone, 0.2 M 2,5-dimethyl-1,4-benzoquinone or a saturated solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone.

The electrode potential was held at + 250 mV vs. Ag/AgCl/sat/KCl and platinum counter electrode was used.

Figure 4A shows the current response on the addition of sarcosine to a solution containing 2,3-dimethoxy-5-methyl-1,4-benzoquinone and sarcosine oxidase. Figure 4B shows the corresponding calibration plot. Figure 5A shows the current response on the addition of sarcosine to a solution containing 2,6-dimethyl-1,4-benzoquinone and sarcosine oxidase, and Figure 5B shows the corresponding calibration plot. Figure 6A shows the current response of 2,5-dimethyl-1,4-benzoquinone/sarcosine oxidase to sarcosine, and Figure 6B shows the corresponding calibration plot.

Example 7

Chronoamperometry was used to show the mediator ability of some compounds in solution to glucose oxidase. The change in current at a glassy carbon electrode was observed on consecutive addition of aliquots of 1 M glucose stock solution to buffer (pH 7.4) containing 100 units/ml glucose oxidase and either 0.2 M 2,5-dimethyl-1,4-benzoquinone, a saturated solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone or a saturated solution of 2-hydroxymethyl-6-methoxy-1,4-benzoquinone.

The electrode potential was held at + 250 mV vs. Ag/AgCl/sat/KCl and platinum counter electrode was used.

Figure 7A shows the current response of 2,5-dimethyl-1,4-benzoquinone/glucose oxidase to glucose, and Figure 7B shows the corresponding

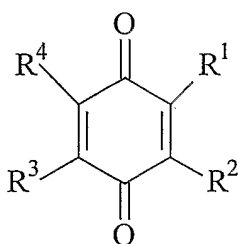
calibration plot. Figure 8A shows the current response of 2,3-dimethoxy-5-methyl-1,4-benzoquinone/glucose oxidase to glucose and Figure 8B shows the corresponding calibration plot. Figure 9A shows the current response of 2-hydroxymethyl-6-methoxy-1,4-benzoquinone/glucose oxidase to glucose, and Figure 5 9B shows the corresponding calibration plot.

CLAIMS

1. An amperometric sensor suitable for determining the concentration of
 5 analyte in a sample, said sensor comprising an oxidase enzyme and a substituted 1,4-
 benzoquinone compound which, in oxidized form, functions as a mediator selective
 for reduced enzyme and has an oxidation potential lower than that of unsubstituted
 1,4-benzoquinone.

2. A sensor according to claim 1, wherein the analyte is glucose and the
 10 enzyme is glucose oxidase.

3. A sensor according to claims 1 to 2, wherein the substituted 1,4-
 benzoquinone compound is of formula (I):



(I)

wherein R^1 , R^2 , R^3 and R^4 are the same or different and at least one of R^1 , R^2 , R^3 and
 15 R^4 is an alkyl group or a phenyl group.

4. A sensor according to claim 3, wherein the alkyl groups have from 1
 to 3 carbon atoms.

5. A sensor according to claim 4, wherein the 1,4-benzoquinone
 compound is chosen from 2,6-dimethyl-1,4-benzoquinone, tetramethyl-1,4-
 20 benzoquinone, methyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone and
 phenyl-1,4-benzoquinone.

6. A sensor according to claims 1 to 2, wherein the 1,4-benzoquinone
 compound is of formula (I) wherein R^1 , R^2 , R^3 and R^4 are the same or different and at
 least one of R^1 , R^2 , R^3 and R^4 is an alkoxy group or a hydroxyalkyl group.

7. A sensor according to claim 6 wherein the alkoxy group or
 25 hydroxyalkyl group have from 1 to 3 carbon atoms.

8. A sensor according to claim 6 or 7, wherein the benzoquinone

compound is chosen from 2,6-dimethoxy-1,4-benzoquinone, 2,3-dimethoxy-5-methyl-1,4-benzoquinone and 2-hydroxymethyl-6-methoxy-1,4-benzoquinone.

9. A cartridge for an amperometric sensor suitable for measuring analyte in a sample, which cartridge comprises an enzyme and a substituted benzoquinone compound as defined in any one of the claims 1 to 8.

10. Use of an amperometric sensor as claimed in any one of claims 1 to 8 for determining the concentration of an analyte in a sample, wherein the enzyme of the sensor reacts with the analyte to produce reduced enzyme which is then regenerated by the mediator.

Fig. 1.

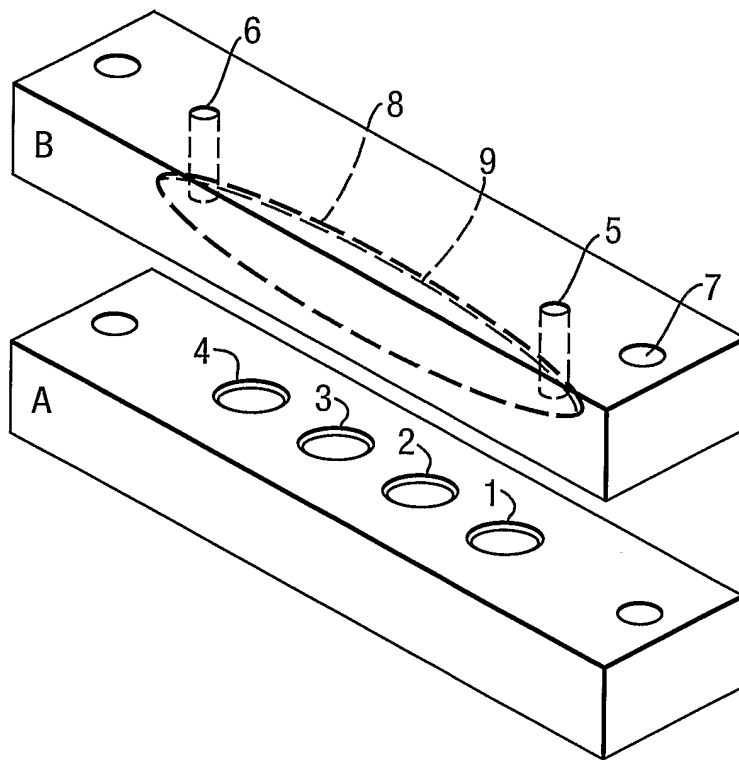


Fig.2A.

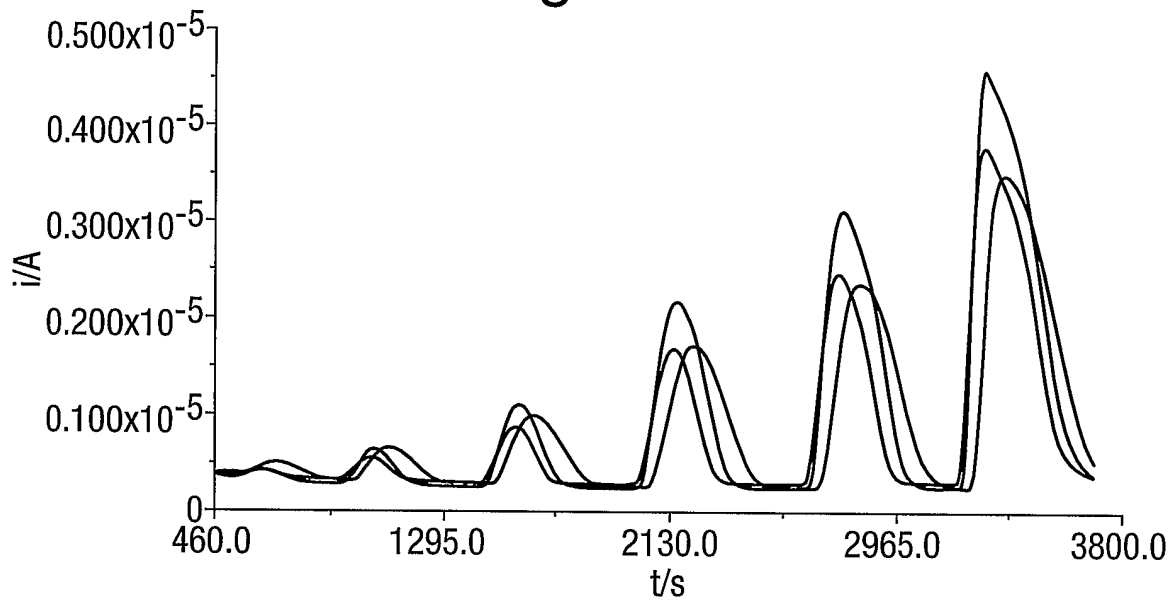


Fig.2B.

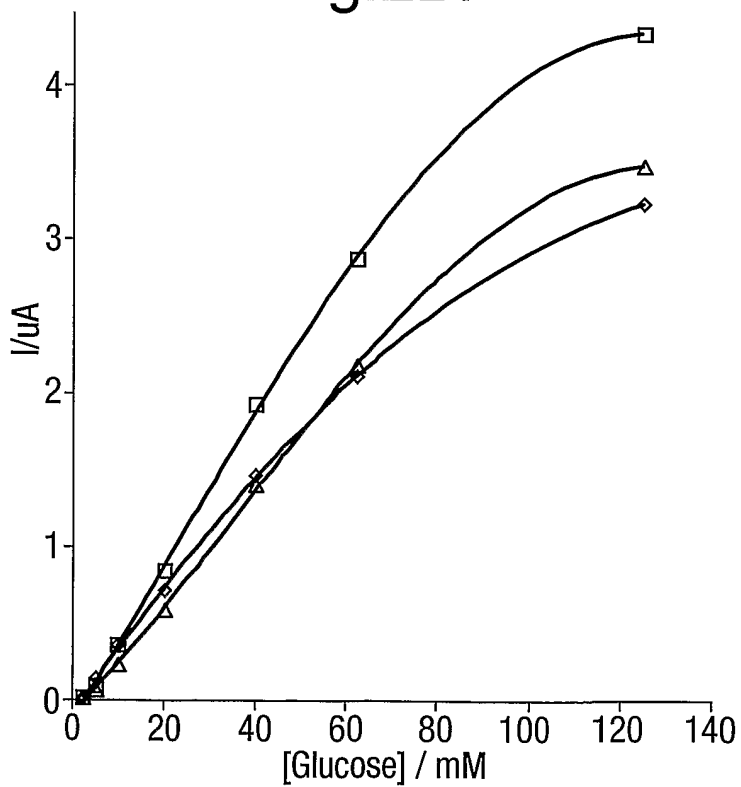


Fig.3A.

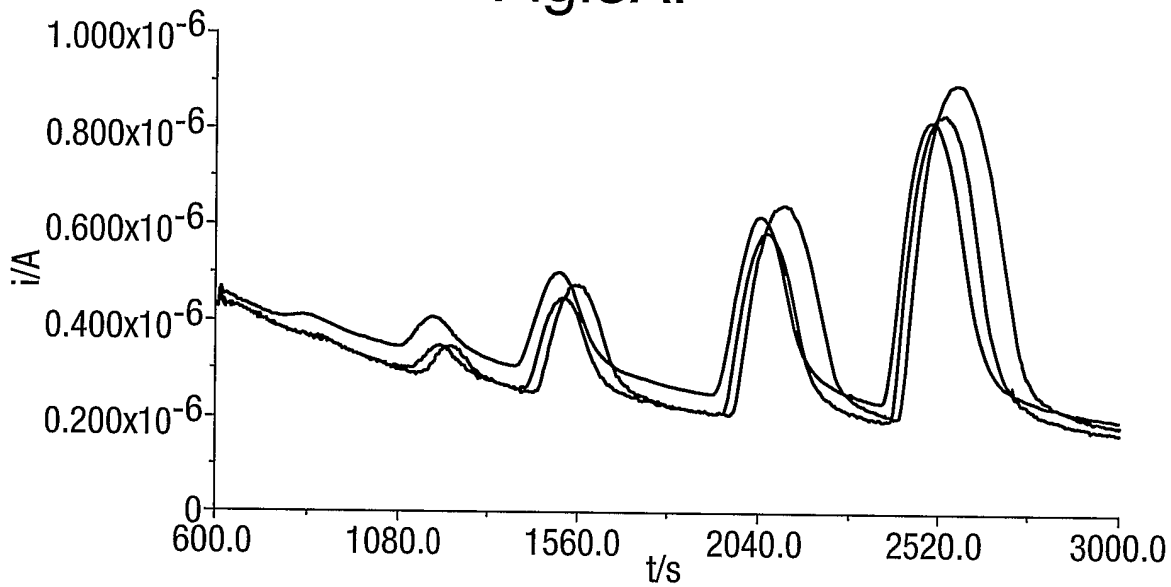


Fig.3B.

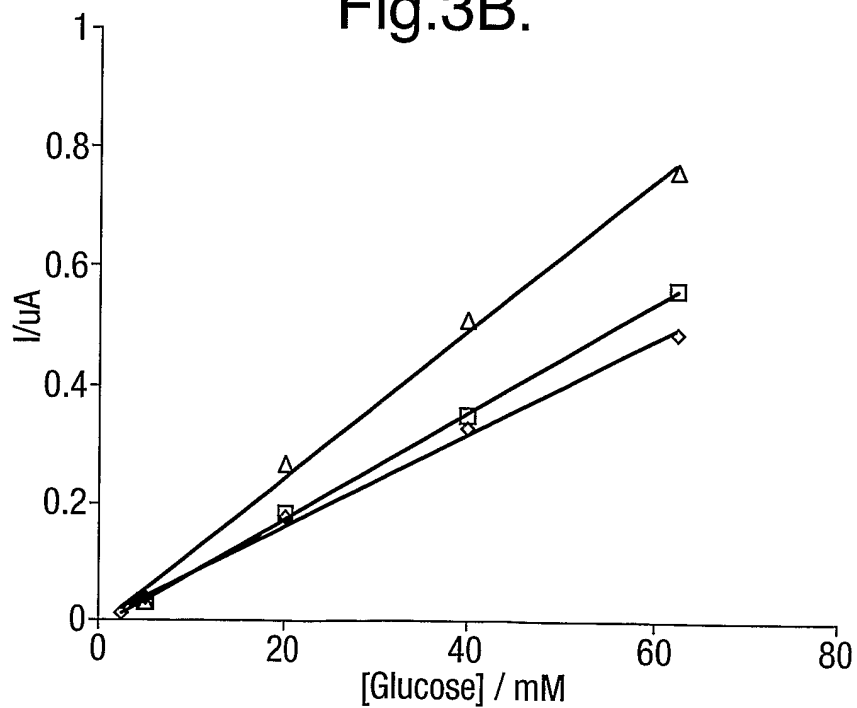


Fig.4A.

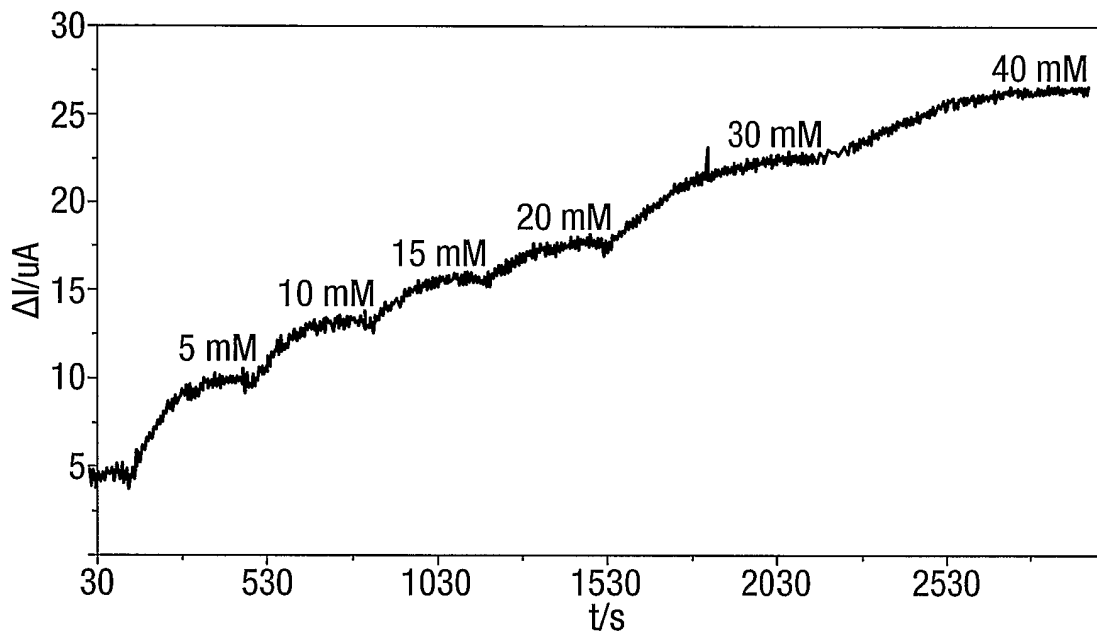


Fig.4B.

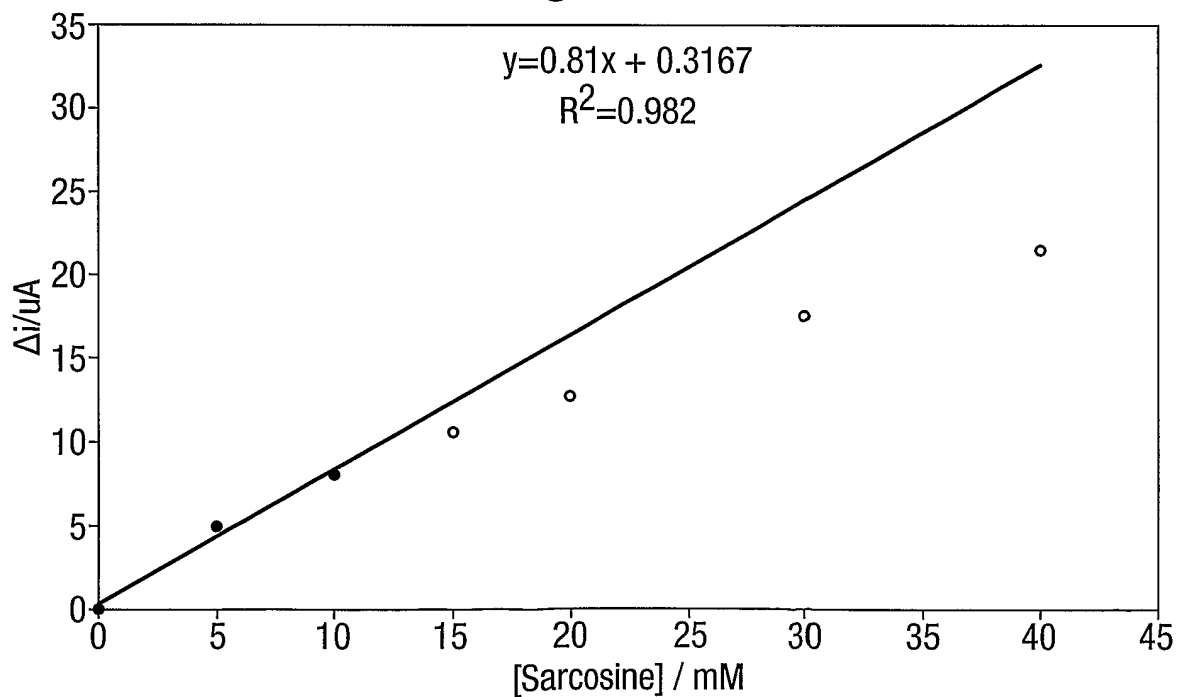


Fig.5A.

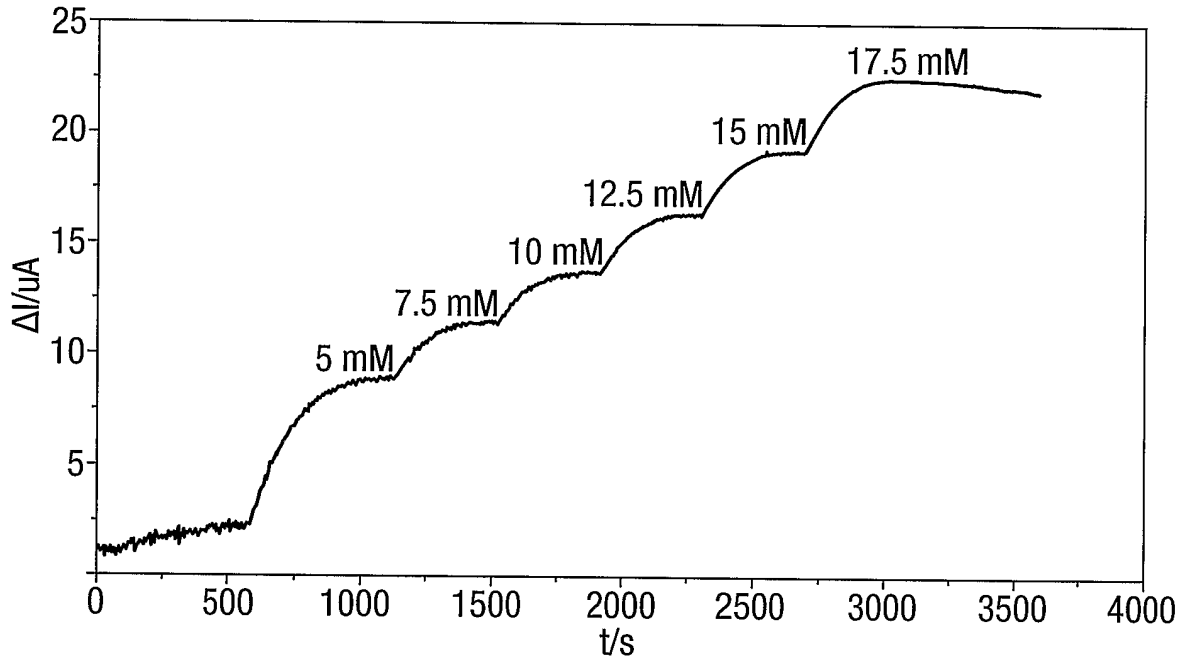
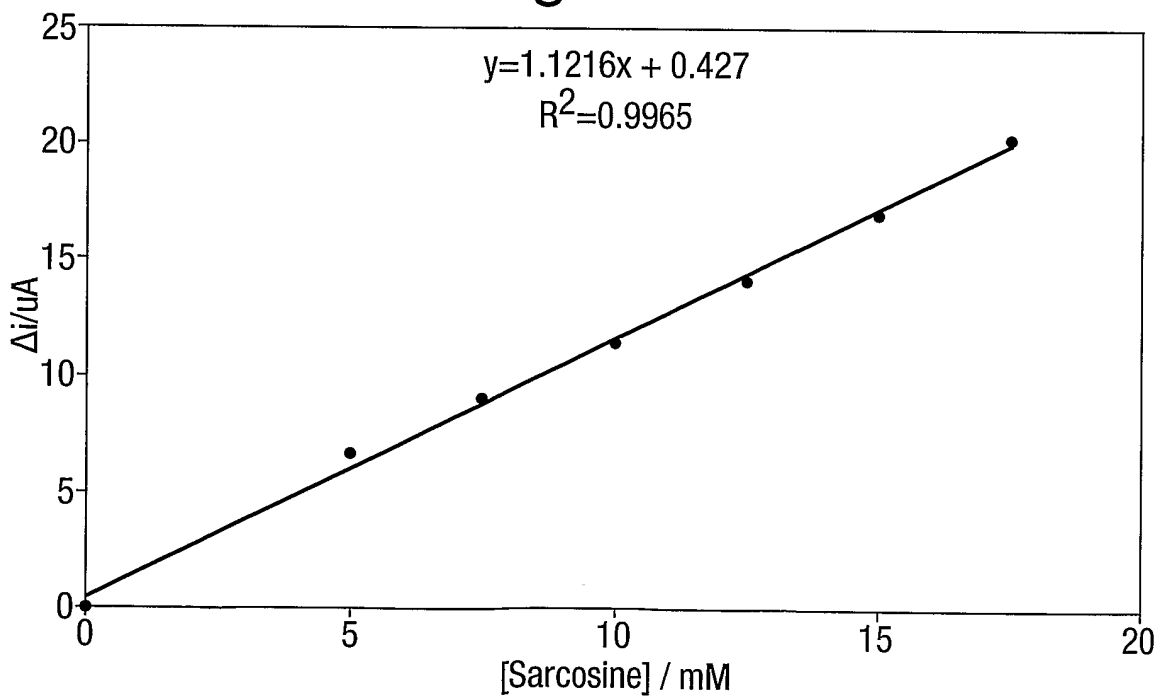


Fig.5B.



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Fig.6A.

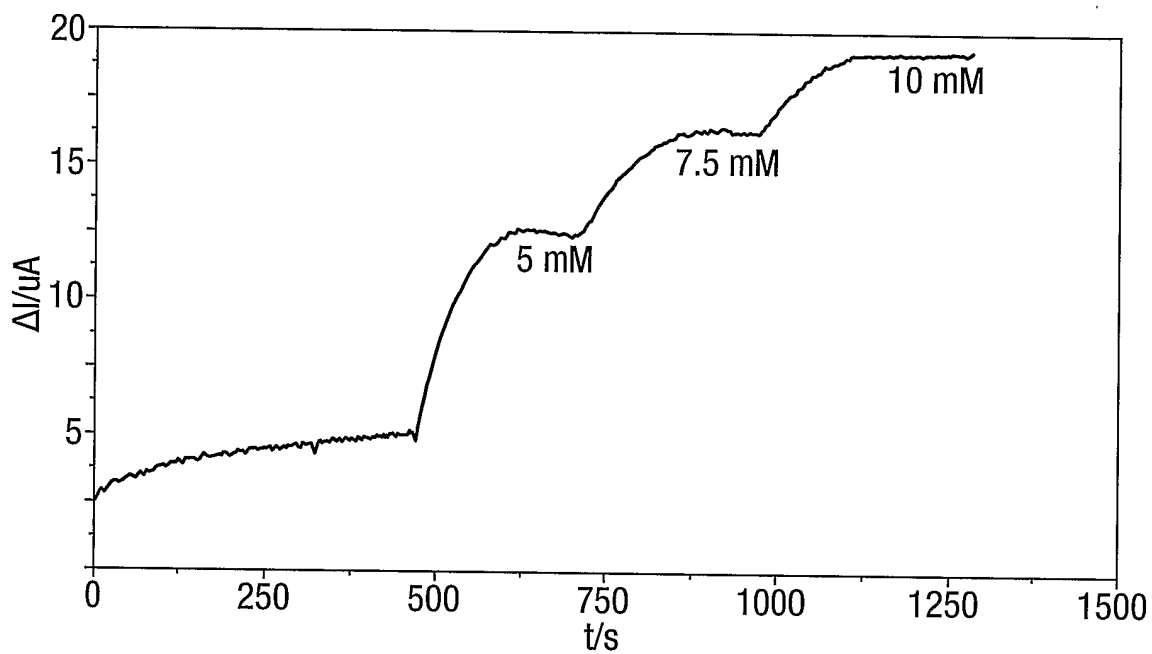


Fig.6B.

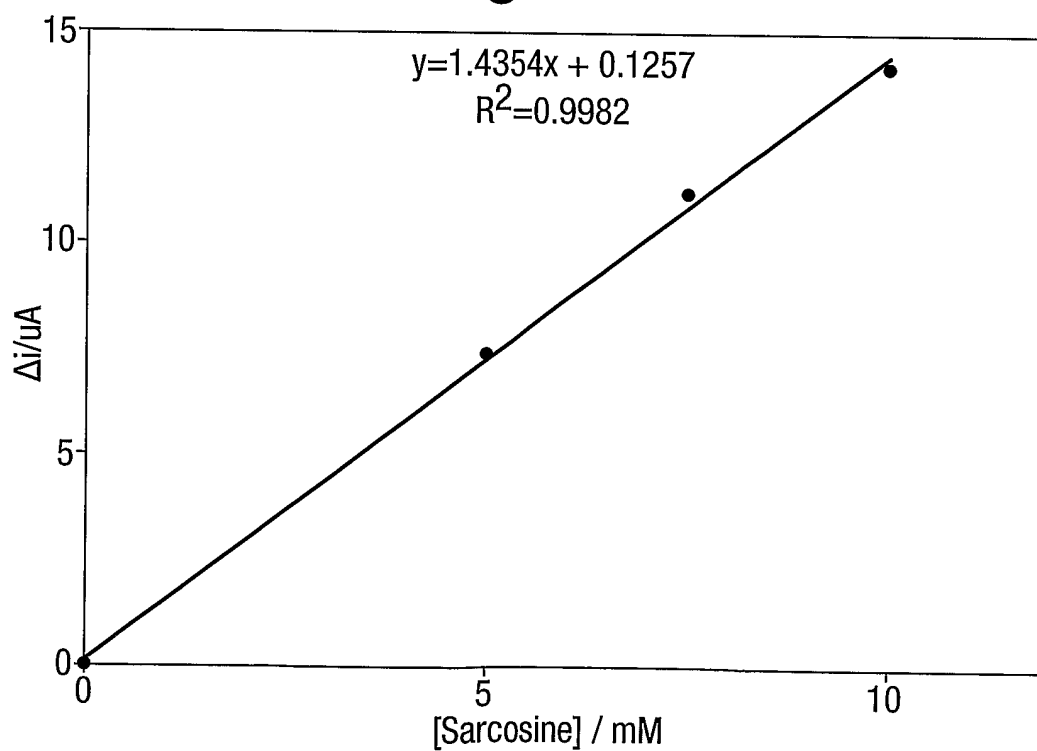


Fig.7A.

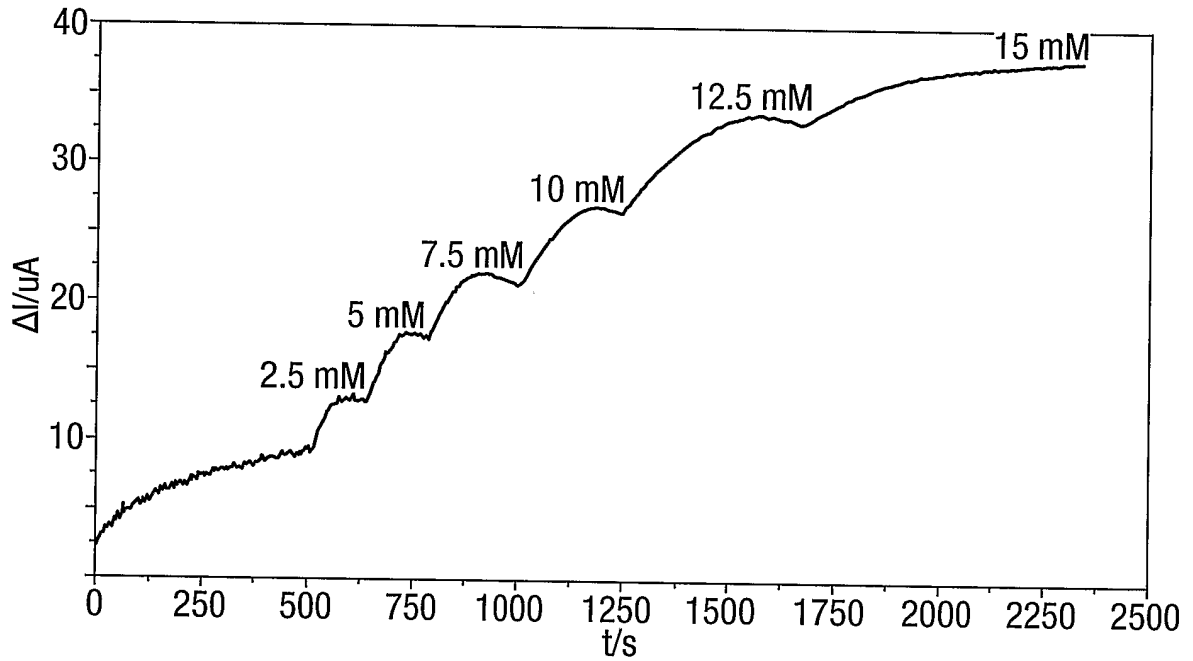


Fig.7B.

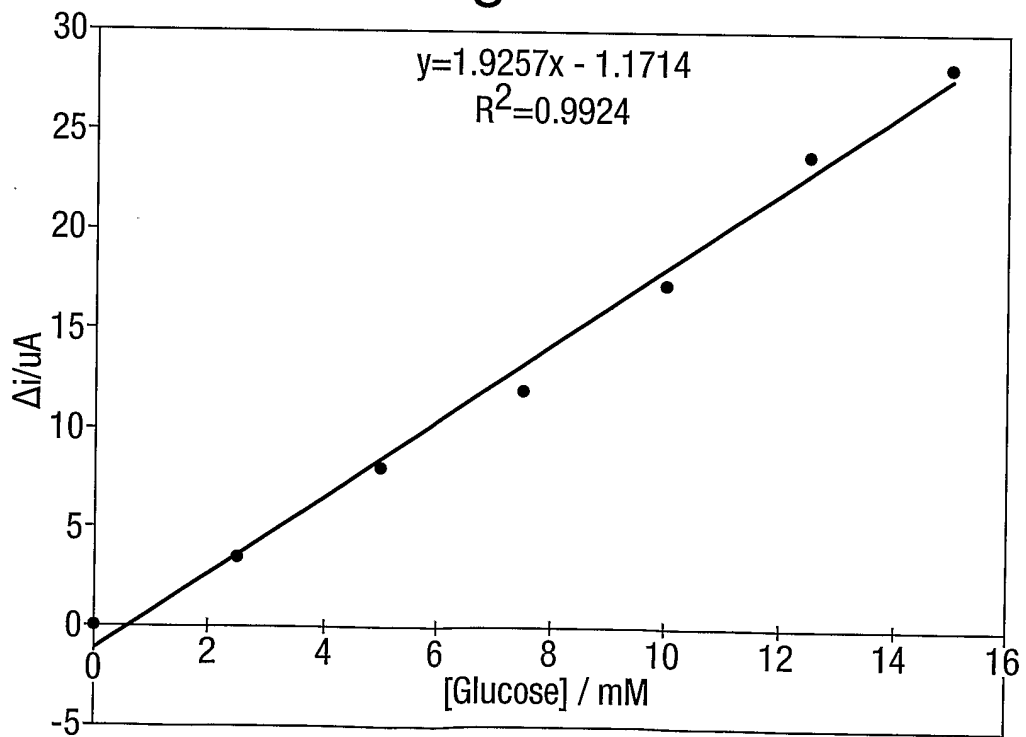


Fig.8A.

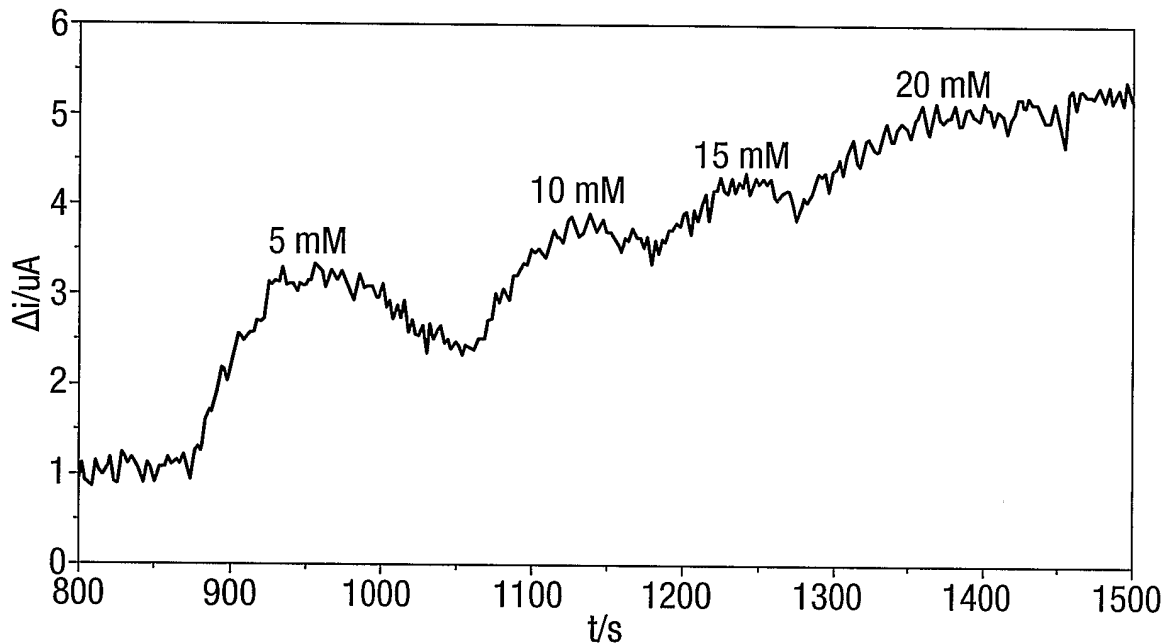


Fig.8B.

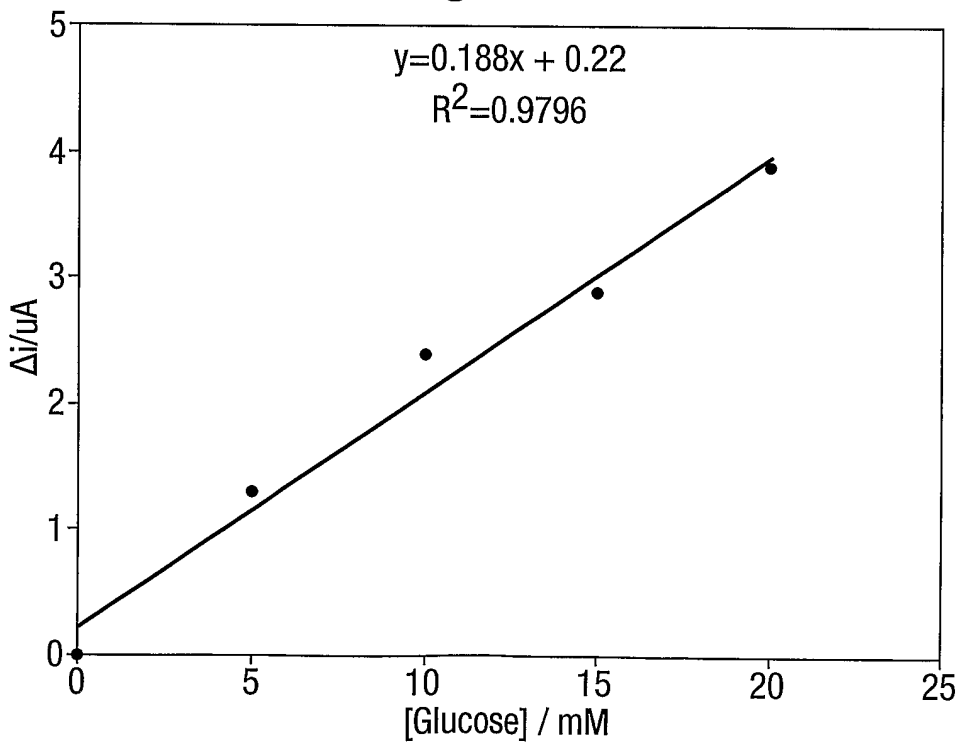


Fig.9A.

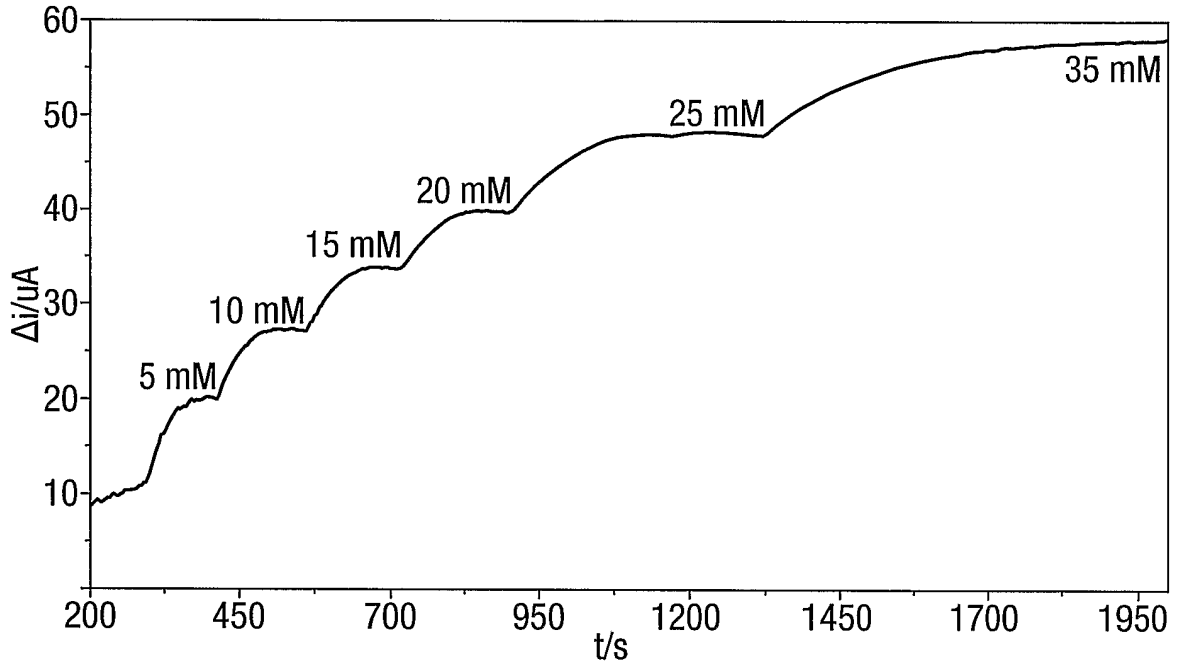
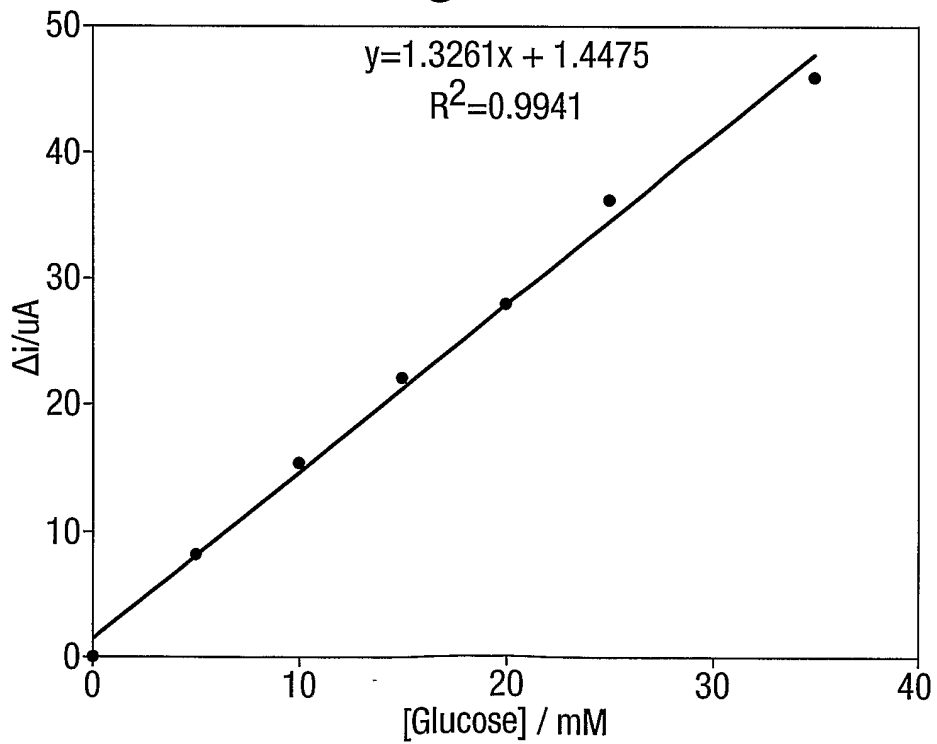


Fig.9B.



INTERNATIONAL SEARCH REPORT

PCT/GB 02/04268

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/00 G01N27/327		
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) IPC 7 C12Q G01N		
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, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 07263 A (NOVONORDISK AS) 30 April 1992 (1992-04-30) page 1, line 2-7 page 5, line 7-31 page 6, line 17 -page 13, line 5 ---	1-10
X	WO 00 20626 A (THERASENSE INC) 13 April 2000 (2000-04-13) page 31, line 30 -page 32, line 2 abstract ---	1,2,6-10
X	US 5 288 636 A (POLLMANN KLAUS H ET AL) 22 February 1994 (1994-02-22) column 5, line 14-68 table 1 abstract --- -/--	1-5,9,10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
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"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 17 December 2002		Date of mailing of the international search report 08. 01. 03
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2260 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer HENRIK ERIKSSON/JA A

INTERNATIONAL SEARCH REPORT

PCT/GB 02/04268

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 385 846 A (KUHN LANCE S ET AL) 31 January 1995 (1995-01-31) column 2, line 37-53 -column 5, line 63-68 ---	1,3-5,9, 10
A	US 4 711 245 A (HILL HUGH A O ET AL) 8 December 1987 (1987-12-08) the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

PCT/GB 02/04268

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9207263	A	30-04-1992	AT 140536 T	15-08-1996
			AU 8725391 A	20-05-1992
			DE 69120956 D1	22-08-1996
			DE 69120956 T2	23-01-1997
			WO 9207263 A1	30-04-1992
			EP 0552223 A1	28-07-1993
			JP 6503472 T	21-04-1994

WO 0020626	A	13-04-2000	US 6338790 B1	15-01-2002
			AU 6420899 A	26-04-2000
			CA 2346415 A1	13-04-2000
			CN 1322254 T	14-11-2001
			EP 1119637 A1	01-08-2001
			JP 2002526759 T	20-08-2002
			WO 0020626 A1	13-04-2000
			US 6299757 B1	09-10-2001
			US 6461496 B1	08-10-2002

US 5288636	A	22-02-1994	US 5508171 A	16-04-1996
			AT 124990 T	15-07-1995
			AU 634863 B2	04-03-1993
			AU 7171691 A	18-07-1991
			CA 2069946 A1	16-06-1991
			DE 69020908 D1	17-08-1995
			DE 69020908 T2	15-02-1996
			EP 0505494 A1	30-09-1992
			ES 2075955 T3	16-10-1995
			JP 3171444 B2	28-05-2001
			KR 171222 B1	18-02-1999
			WO 9109139 A1	27-06-1991

US 5385846	A	31-01-1995	CA 2140903 A1	04-12-1994
			DE 69422687 D1	24-02-2000
			DE 69422687 T2	29-06-2000
			EP 0653068 A1	17-05-1995
			ES 2142946 T3	01-05-2000
			JP 8500190 T	09-01-1996
			WO 9429731 A1	22-12-1994

US 4711245	A	08-12-1987	AU 564494 B2	13-08-1987
			AU 2775184 A	31-01-1985
			AU 564495 B2	13-08-1987
			AU 2775284 A	31-01-1985
			AU 569076 B2	21-01-1988
			AU 2775384 A	08-11-1984
			AU 580257 B2	12-01-1989
			AU 2775484 A	08-11-1984
			CA 1219040 A1	10-03-1987
			CA 1223638 A1	30-06-1987
			CA 1218704 A1	03-03-1987
			CA 1220818 A1	21-04-1987
			DE 3483019 D1	27-09-1990
			DE 3483170 D1	18-10-1990
			EP 0125867 A2	21-11-1984
			EP 0125136 A2	14-11-1984
			EP 0125137 A2	14-11-1984
			EP 0125139 A2	14-11-1984
			JP 60017360 A	29-01-1985

INTERNATIONAL SEARCH REPORT

PCT/GB 02/04268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4711245	A	JP 60017346 A	29-01-1985
		JP 60017347 A	29-01-1985
		JP 60017345 A	29-01-1985
		US 4758323 A	19-07-1988
		AU 572138 B2	05-05-1988
		CA 1226036 A1	25-08-1987
		DE 3485554 D1	16-04-1992
		DE 3486221 D1	04-11-1993
		DE 3486221 T2	27-01-1994
		EP 0127958 A2	12-12-1984
		EP 0351891 A2	24-01-1990
		EP 0351892 A2	24-01-1990
		JP 3026430 B2	27-03-2000
		JP 9325127 A	16-12-1997
		JP 7072727 B	02-08-1995
		JP 60017344 A	29-01-1985
		JP 3103813 B2	30-10-2000
		JP 2000055865 A	25-02-2000
		US 5682884 A	04-11-1997
		US 5509410 A	23-04-1996
		US 5727548 A	17-03-1998
		US 5820551 A	13-10-1998
