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(54) **Title**: CARBON NANOTUBE-MODIFIED ELECTRODE

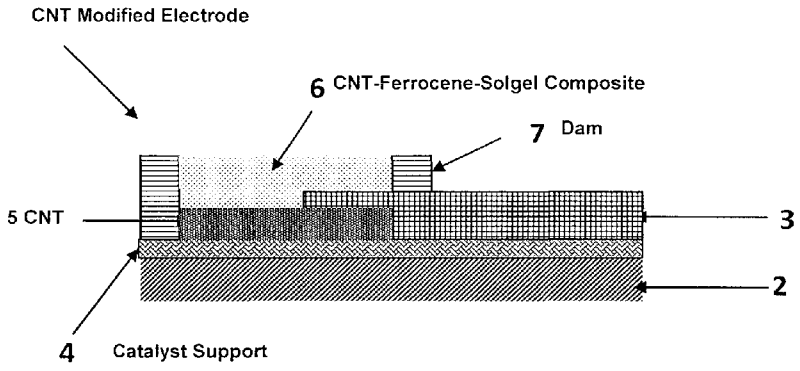

FIGURE 1

(57) **Abstract**: A non-enzymatic biosensor based on selective voltammetry using functionalized hybrid solgel in its electrode is proposed. A preferred embodiment of the electrode comprises a conductor layer (3) which is disposed on a substrate (2), carbon nanotubes (CNT) (5) which are disposed on at least a portion of the conductor layer (3) such that the CNT's basal (5a) and distal ends (5b) are respectively disposed in between the conductor layer (3) and a CNT-ferrocene solgel composite (6) deposited onto the distal end (5b) of the CNT (5) within a dam structure (7). The conductor layer (3) may be silver, platinum, gold or carbon. Preferably, a catalyst support (4) is deposited on the substrate (2) and a liquid-form nickel catalyst is deposited on the catalyst support. The CNT-ferrocene solgel composite is made by vigorously mixing (by weight) CNT 10-35%, tetraethylorthosilicate 20-40%, methyltri-ethoxysilicon 20-40%, phenyltriethoxysilicon 20-40%; and ferrocene 0.1 to 10%. The mixture is sonicated for about 1 minute and then left to stand for about 20 hours.

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Carbon Nanotube-modified Electrode

TECHNICAL FIELD

This invention concerns electrodes of biosensors for detecting analytes, including body metabolites such as blood glucose and uric acid. In particular, the electrodes are configured from modified carbon nanotubes (CNT), which includes an electron transfer agent for redox reaction with the metabolite.

BACKGROUND ART

Enzyme electrodes are widely used for detection of metabolites in body fluids. The level of these analytes detected in human blood or urine is used to diagnose diseases such as diabetes, renal failure and cardiovascular problems. The main advantage of enzyme-based electrodes is the high selectivity nature of enzymatic reaction - enzyme selectively catalyzes conversion of a target substance to the corresponding product. However, there are also many disadvantages in using enzymes. This is due to enzymes being proteins and the specificity of enzymatic transformation depends on complex three-dimensional interaction between the amino acid units in the enzyme.

These interactions give secondary and tertiary structures of the enzymes, and the structures may start to change or unravel once the enzyme is outside of living cells. Consequently, enzymatic reactions deteriorate over time and this causes inaccurate biosensor data. Moreover, enzymes contain numerous reactive sites that may be disrupted during immobilization step whereby the enzyme is attached to the electrode.

Reactive residues in enzyme such as thiol, alcohol, amine and carboxylic acid take part in most organic transformations, and this can significantly change the enzyme folding mechanism, three-dimensional structure and reaction, which negatively affect the sensor accuracy. Furthermore, enzymes are normally stored under chilled condition in order to slow down denaturing process, and this becomes

a serious limitation in the deployment of mass-produced biosensors. Enzyme denaturing also seriously limits the sensor lifetime.

Electrodes for detecting analytes, including body metabolites are known in the art to be fabricated with carbon nanotubes (CNT) which have been configured or arranged in a manner to provide the required electrical response to specific analytes. CNT may, for example, be grown via chemical vapour deposition (CVD) on catalytically prepared surface and the resulting carbon structure typically provides superior ratio of surface area to volume, good electrical conductivity, high mechanical strength, organic functionalization and electrochemical inertness as an electrochemical transducer.

With good device design and choice of fabrication techniques, CNT can be grown directly on a suitable substrate to function as the main backbone to construct a voltametric working electrode. For example, in U.S. Patent No. 7,013,708 (Cho), the carbon nanotubes may be doped with boron, nitrogen and oxygen atoms so that the necessary electrical response may be had upon the electrode's contacting with carbon monoxide. In U.S. Application No. 2007/0145356 (Amlani), the CNT are configured as interlaced digits so that electrical conductivity between the pair of inter-digitated electrodes could be varied according to the analyte solution.

It is also know in the art for the CNT to be modified with ferrocene (typically represented by simplified formula "$Fe(Cp)_2$") due to its redox properties although it is also known as a catalyst for CNT production and as ligand scaffold for support purposes. For example, in A.J. Saleh Ahammad *et al.*, "*Electrochemical Sensors based on Carbon Nanotubes*", **Sensors** 2009:9, pp 2289-2319, published 30 March 2009 (retrieved from www.mdpi.com/journal/sensors), a ferrocene-filled single-walled CNT has been cited for use as an electron transfer mediator for the electrode. In You Wang, *et al.* "*Electrochemical sensors for clinical analysis*", **Sensors** 2008:8, pp. 2043-2081, published 27 Mar 2008, CNT with ferrocene as electron transfer mediator has also been cited.

The above publications also make references to sol-gel systems as precursor mixtures in conjunction with enzyme and other biomolecules for redoxing the specific metabolite or analyte. These solgel composite materials are for the purpose

of entrapping and immobilizing enzymes necessary for specifically reacting with the designated analytes. As with most enzyme-based systems, the biosensor comprised of such electrode are usually complex in terms of processing the analytes and is limited in terms of shelf-life when stored at ambient conditions.

It would be advantageous for a CNT-modified electrode for detecting metabolites to be so configured without enzymes so that the processing is simplified. It is also desirous that a composite material be developed such that the electrical response to different metabolites may be discerned in conjunction with the CNT-ferrocene modified electrode in the absence of enzymes and other biodegradable molecules.

SUMMARY OF DISCLOSURE

We now propose a non-enzymatic biosensors comprising CNT-modified electrode for detecting and measuring concentrations of metabolites of interest in body fluids, such as blood, urine or sweat, i.e. 3 of the most important bodily fluids to be analysed for medical diagnosis. Without enzymes, a biosensor with the proposed electrode may have its analysis process simplified and allow for longer term shelf life at ambient conditions. Our proposed sensors ought to be manufactured at low cost and good accuracy so that devices may be made for consumer or patient's own or home use such that the data obtained therefrom may be shared with, collected or used by the patient's doctor or hospital, and long term profile of the consumer may be retrievably stored.

Our proposed non-enzymatic biosensor working concept is based on selective voltammetry using functionalized hybrid solgel. In the first aspect of our invention, our electrode configuration comprises a conductor layer which is disposed on a substrate, carbon nanotubes (CNT) which are disposed on at least a portion of the conductor layer such that the CNT's basal and distal ends are respectively disposed in between the conductor layer, and a CNT-ferrocene solgel composite deposited onto the distal end of the CNT, wherein the CNT-ferrocene composite provides a surface for redox reaction with the metabolite. The CNT-ferrocene solgel composite may be contained, preferably by a dam structure, at the distal end of the

CNT, while leaving a surface available for chemical contact by a metabolite.

While the conductor layer provides electrical contact between the electrode and a readout circuitry, it is preferably disposed on the substrate by screen printing, electrodeposition, or electroless deposition of silver, platinum, gold or carbon. The catalyst support may be chosen from any one of silicon oxide, silicon nitride, titanium nitride, indium tin oxide or aluminium oxide, and provided on the conductor layer for carbon nanotubes (CNT) to be grown thereon. Preferably, a nickel catalyst in liquid form is deposited on the catalyst support.

In the second aspect, our proposed electrode may be fabricated by depositing a catalyst support layer onto a substrate, applying a catalyst on said catalyst support layer, growing carbon nanotubes (CNT), depositing a conductor layer which makes electrical contact and overlaps with the grown CNT, forming dam structure in a manner for containing a space over said grown CNT, dispensing a CNT-ferrocene solgel composite mixture into said dam structure over said CNT.

Preferably, the catalyst support layer is depositing via screen printing through a stencil mask and may comprised of a thin silicon oxide layer of about 50 nm, patterned with circular shape of 1 mm diameter or square shape of 1 mm × 1 mm with dry oxidation process on a 15.2 cm (6-inch) silicon wafer. The CNT may be grown by chemical vapour deposition (CVD) process, preferably under flow of acetylene and ammonia gasses at temperature in the range of 600 – 850°C and chamber pressure of 1 Torr.

The dam structure may be formed by epoxy paste being dispensed, including by screen-printing and cured at 90° – 120°C for a period from 30 – 90 minutes. The dispensed CNT-ferrocene solgel composite mixture is cured at 80° – 120°C for at least 5 minutes and allowed to cool to room temperature for at least an hour, and preferably dried under continuous flow of nitrogen gas for 1 hour.

In the third aspect, the sol-gel composite for use in an electrode according to our invention may be prepared by vigorously mixing a carbon-based electrochemical-variable transducing medium, a solgel starting material, an alkoxy cross-linker, a dispersant cross-linker and an electron transfer mediator. Preferably,

each of these constituents is a carbon nanostructure material, a tetraalkyl orthosilicate, alkylalkoxysilane, a trifunctional alkoxysilane, and a metallocene. More preferably, the respective constituents are CNT, ferrocene, tetraethylorthosilicate, methyltriethoxysilane and phenyltriethoxysilane which are mixed in a solution of hydrochloric acid, stirred vigorously at room temperature and left in a tightly-capped vial for at least an hour.

Most preferably, the constituents comprise by weight CNT 10-35%, tetraethylorthosilicate 20-40%, methyltriethoxysilicon 20-40%, phenyltri-ethoxysilicon 20-40%; and ferrocene 0.1 to 10%, which are mixed in 0.05 M to 1 M hydrochloric acid prepared with deionized water. Preferably still, the ratio of tetraethylorthosilicate : methyltriethoxysilane : phenyltriethoxysilane are first mixed in equal proportions in 0.1 M hydrochloric acid and 10% (by weight) single-walled CNT and 1% (by weight) ferrocene is added to the mixture which is sonicated for about 1 minute and the sonicated composition left to stand for about 20 hours. Our solgel composite has distinct oxidation potentials for different metabolites which are discernible from voltametric analysis.

LIST OF ACCOMPANYING DRAWINGS

The drawings accompanying this specification, as listed below, may provide a better understanding of our invention and its advantages when referred to, with the detailed description that follows, as exemplary and non-limiting embodiments of our method, in which:

FIGURE 1 shows a generalized, cross-sectional view of the CNT-ferrocene modified electrode according to our invention.

FIGURE 2 illustrates a schematic view of the oxidation of uric acid at a CNT modified electrode according to our invention;

FIGURE 3 embodies schematic reactions in the detection of uric acid and ascorbic acid by an electrode according to our invention; and

FIGURE 4 is a graph representing voltammetric plots of glucose at 3 concentrations as detected by a CNT modified electrode according to our invention;

FIGURE 5 shows a graph plotting oxidation current against concentration of glucose as detected with an electrode according to our present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A general embodiment of our proposed CNT-ferrocene modified electrode is shown in the form of a schematic, cross-sectional view in FIGURE 1 wherein our proposed electrode has the following configuration. Broadly speaking, our proposed electrode may be fabricated with the following process steps which generally comprises of:

- depositing a catalyst support layer (4) onto a substrate (2);
- applying a catalyst on said catalyst support layer (4);
- growing carbon nanotubes (CNT);
- depositing a conductor layer which makes electrical contact and overlaps with the grown CNT;
- forming dam structure in a manner for containing a space over said grown CNT;
- dispensing a CNT-ferrocene solgel composite mixture into said dam structure over said CNT.

A substrate (2) is first provided whereupon a conductor layer (3) is disposed. The substrate (2) may be an insulating board and may be one of the following materials but not limited to silicon wafer, glass, fibreglass, epoxified woven glass fibres, printed circuit board, including FR4. The substrate (2) provides mechanical strength to the overall electrode structure in addition to providing a surface for good adhesion to the conductor layer (3) upon which carbon nanotubes (CNT) may be grown. The conductor (3) generally provides electrical contact between the CNT and the electrode's readout circuitry. The conductor (3) layer may be disposed on the substrate (2) by any method, including one or combination of screen printing, electrodeposition or non-electrodeposition of silver, platinum, gold or carbon.

With reference to FIGURE 2 which shows, in longitudinal view, the profile of a single tube of CNT, the particular CNT is shown disposed on the conductor such that the CNT's basal ends (5a) are electrically connected to the conductor layer (3) while the distal ends (5b) are electrically connected to a CNT-ferrocene solgel composite. The CNT functions as an electrochemical transducer whereas the CNT-ferrocene solgel composite material serves or provides a surface for redox reaction with metabolites by selective voltametric oxidation. There are many CNT growth mechanism that have been developed such as template-assisted synthesis, laser ablation, chemical vapour deposition (CVD), electrochemical deposition (ECD) or vapour-liquid-solid (VLS) approach. The CNT is preferably grown by chemical vapour deposition (CVD) process. In one specific embodiment, the CVD process occurs under flow of acetylene and ammonia gasses at temperature in the range of 600 – 850°C and chamber pressure of 1 Torr.

A catalyst support (4) for carbon nanotubes (CNT) growth is preferably provided on the conductor (3) layer. The catalyst support (4) may be chosen from any one of silicon oxide, silicon nitride, titanium nitride, indium tin oxide or aluminium oxide. More preferably, a layer of nickel catalyst – about 5 nm thickness – may be deposited on the catalyst support. The nickel catalyst is preferably in liquid form, such as nickel nitrate in liquid metal droplets form ($Ni(NO_3)_2.6H_2O$).

Such catalyst support materials functions to catalytically treat the conductor (3) surface for *in situ* thermal growth of CNT. The catalyst support layer may be depositing via screen printing through a stencil mask. As specific embodiments, the catalyst support layer (4) may preferably comprise of a thin silicon oxide layer of about 50 nm, patterned with circular shape of 1 mm diameter or square shape of 1 mm × 1 mm with dry oxidation process on a 15.2 cm (6-inch) silicon wafer.

The dam structure is preferably formed by epoxy paste being dispensed, including by screen-printing. In one specific embodiment, the dam structure formed is cured at 90° – 120°C for a period from 30 – 90 minutes.

The CNT-ferrocene solgel composite is preferably contained at the distal end of the CNT by a dam structure (7) which leaves a surface area of the composite

material available for chemical contact by a metabolite. The sol-gel composite (6) may generally comprises a carbon-based variable electrochemical transducing medium, a solgel starting material, an alkoxy cross-linker, a dispersant cross-linker and an electron transfer mediator. Preferably, the respective constituents of the solgel composite are carbon nanostructure material, a tetraalkyl orthosilicate, an alkylalkoxysilane, a trifunctional alkoxysilane and a metallocene. More preferably, the CNT-ferrocene solgel composite (6) comprises (by weight):

- CNT – 10-35%;
- tetraethylorthosilicate – 20-40%;
- methyltriethoxysilane – 20-40%;
- phenyltriethoxysilane – 20-40%; and
- ferrocene – 0.1 to 10%.

It should be noted that in this specification, the suffix *~silicon*, *~silicate* and *~silane* have been used interchangeably to designate the above compounds which are considered silicon analogs of alkane hydrocarbons. Thus, tetraethylorthosilicate is referred to synonymously with tetraethylorthosilane, methyltriethoxysilicon with methyltriethoxysilane, phenyltriethoxysilane with phenyltriethoxysilicon, and so on.

The aforesaid constituents may be mixed and prepared into the solgel composite by mixing the CNT, ferrocene, tetraethylorthosilicate, methyltriethoxy silane and phenyltriethoxysilane in 0.05 M to 1 M solution of hydrochloric acid in deionized water. The mixture may then be stirred vigorously at room temperature and then left to stand in a tightly-capped vial for at least an hour.

The ready-mixed CNT-ferrocene solgel composite may then be dispensed into the dam structure (7) to be cured. An optimal and preferred curing condition is 80° – 120°C for at least 5 minutes and allowed to cool to room temperature for at least an hour. Thereafter, the dispensed CNT-ferrocene solgel composite mixture may be dried or cured under continuous flow of nitrogen gas for at least 1 hour.

In one specific embodiment, the ratios of tetraethylorthosilicate to methyltriethoxysilane to phenyltriethoxysilane being mixed are in equal proportions in 0.1 M hydrochloric acid prepared in deionized water. The mixture is than stirred

for 4 hours at room temperature. To this mixture of silanes is added 10% by weight single-walled CNT and 1% by weight ferrocene. The mixture is then mixed well by sonicating the composition for about 1 minute and then left to stand for 20 hours.

Certain aspects of our invention may be further described in the following non-limiting examples.

EXAMPLE 1
Preparation of thermally grown carbon nanotubes

A stencil mask is used to directly deposit a support layer (10 – 50 nm ITO (indium tin oxide), TiN – titanium nitride) followed by the CNT catalyst (0.5 – 5 nm Ni, Co or Fe) onto a substrate (Si, glass, polymer). The deposition is performed either via physical vapour deposition or liquid chemical based. As a result of the stencil mask, predefined patterns of the CNT sensor electrode (~ 1 x 1 mm) and alignments marks are produced onto the substrate. The substrate is then subjected to chemical vapour deposition (CVD) growth within a temperature deposition range of 600 – 850°C resulting in the formation of CNTs on the patterned area.

The full conditions of the CNT deposition includes a flow of 1:3 ratio of acetylene to ammonia gases, chamber pressure of 1 Torr and low frequency plasma power of 50 – 100W being applied. After CVD, a separate stencil mask is then used as an overlay to deposit metal contacts (~ 2 μm thickness of Al) along the perimeter of the CNT electrode. Following this step another stencil mask is applied to deposit the hybrid solgel via spraying (~ 1 μm thickness) that encapsulates the overall CNT electrode.

EXAMPLE 2
Preparation of carbon nanotubes-ferrocene-solgel composite

Tetraethyl orlhosilicate (TEOS, 450 μl), 450 μl methyltriethoxysilane (MTES), 280 μl of deionized water (DIW) and 20 μl of 0.1 M hydrochloric acid are mixed in a glass vial or round-bottom flask. The mixture was stirred for 4 hours until a clear solution was achieved. Then, 200 μl of the homogenous mixture was mixed with 0.3 g of single-walled carbon nanotubes and 0.3 g of ferrocene and the mixture sonicated for 10 minutes. The cocktail of CNT-ferrocene-solgel composite (3

μl) was dispensed into the epoxy dam on the thermally grown carbon nanotubes. The CNT-ferrocene-solgel composite was dried under continuous flow of nitrogen gas for 1 hour.

EXAMPLE 3
Carbon nanotube modified electrode glucose sensor

The CNT-ferrocene-solgel modified electrode was characterized as a glucose sensor in a conventional three-electrode cell with a platinum wire counter electrode and Metrohm Ag/AgCI double junction reference electrode using low-noise, low current potentiostat/galvanostat such as the Metrohm Autolab PGSTAT 128N. The three electrodes were immersed into 3 glucose calibration solutions; 2.5 mM, 5 mM and 10 mM glucose buffered by phosphate at pH 7. Linear sweep voltammetry were conducted for the above 3 solutions of glucose from -1.0 V to +1.0 V with a potential sweep rate of 100 mV sec^{-1}. Voltammetric oxidation current peaks (I) at about 0.3 V were observed in the linear sweep voltammetry plots of CNT modified electrode for each of the 3 concentrations of glucose, as shown in FIGURE 4.

Table 1 shows oxidation peaks (I) at voltage of about 0.3 V for the respective 3 glucose concentrations. FIGURE 5 shows the plot of these data.

Glucose [mM]	I (mA)
2.5	0.0715
5	0.0592
10	0.0481
M	-0.003
C	0.077
r^2	0.9520

As will be apparent to a person skilled in the art, a biosensor with CNT-modified electrode fabricated using the solgel technique as described herein may be used to selectively oxidize molecules of biomedical interest such as glucose, uric acid and cholesterol, and thus provides alternative to the conventional enzyme-based electrodes. As a specific example as shown in FIG. 2, the CNT-modified electrode doped with ferrocene according to our invention may be used to selectively oxidize uric acid to allantoin, which may be detected by a simple and reproducible

voltammetric setup. This detection method may be adapted to selectively detect metabolites and other biomolecules at low concentrations and may thus be configured for diverse bio-medical applications.

The simultaneous and selective sensing and measurement of multiple analytes may also be achieved based on the voltametric oxidation schemes of dilute solutions of uric acid and ascorbic acid using our proposed solgel electrode as illustrated schematically in FIGURE 3. We have also found that with our CNT-modified electrode doped with ferrocene in hybrid sol-gel composite, the detection of uric acid and ascorbic acid results in two distinct voltammetric oxidation peaks and may be done with good reproducibility. Each of the distinct oxidation peaks is the result of the different oxidation potential (E1) for uric acid and oxidation potential (E2) for ascorbic acid. Accordingly, the solgel ccomposite material fabricated according to our invention provides distinct oxidation potentials for different metabolites coming into contact with it. The different oxidation potentials thus enable the voltammetric value of the different metabolite being oxidized to be detected and measured by the biosensor. The biosensor can also be cleaned subsequently and reused for many times.

Apart from the afore-described embodiments of our electrode configuration, methods of fabrication (such as methods of growing the CNT) and methods for making the sol-gel composite, there are many aspects or advantages of our invention which may be presented in other forms, variations, substitution or modifications to the many compounds, compositions and substances suggested herein that would be obvious to a skilled person without departing from the essence and working principles of the invention. Such suitable variations, alternates, analogs or equivalents are to be considered as falling within the letter and scope of the following claims.

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CLAIMS

1. An electrode for detecting at least a metabolite, said electrode comprising:
   - a conductor layer (3), which is disposed on
   - a substrate (2);
   - carbon nanotubes (CNT) (5) which are disposed on at least a portion of said conductor (3) layer, such that said CNT's (5) basal and distal ends (5a, 5b) are respectively disposed in between said conductor layer (3) and
   - a CNT-ferrocene solgel composite (6) deposited onto said distal end of said CNT (5)

   wherein said CNT-ferrocene composite (6) provides a surface for redox reaction with said metabolite.

2. An electrode for detecting a metabolite according to Claim 1 wherein the CNT-ferrocene solgel composite is contained at the distal end of the CNT.

3. An electrode for detecting a metabolite according to Claim 2 wherein dam structure (7) is provided to contain the CNT-ferrocene solgel composite (6) on the distal end (5b) of the CNT while leaving a surface available for chemical contact by a metabolite.

4. An electrode for detecting a metabolite according to Claim 1 wherein the solgel composite (6) comprises a carbon-based electrochemical-variable tranducing medium, a solgel starting material, an alkoxy cross-linker, a dispersant cross-linker and an electron transfer mediator.

5. An electrode for detecting a metabolite according to Claim 1 wherein the solgel composite (6) comprises a carbon nanostructure material, a tetraalkyl orthosilicate, a trifunctional alkoxysilane and a metallocene.

6. An electrode for detecting a metabolite according to Claim 1 wherein the CNT-ferrocene solgel composite (6) comprises (by weight):
   - CNT – 10-35%;
   - tetraethylorthosilicate – 20-40%;

- methyltriethoxysilane – 20-40%;
- phenyltriethoxysilane – 20-40%; and
- ferrocene – 0.1 to 10%.

7. An electrode for detecting a metabolite according to Claim 1 wherein the conductor (3) layer provides electrical contact between the electrode and a readout circuitry.

8. An electrode for detecting a metabolite according to Claim 1 wherein the conductor (3) layer is disposed on the substrate (2) by any one or combination of screen printing, electrodeposition, or electroless deposition of silver, platinum, gold or carbon.

9. An electrode for detecting a metabolite according to Claim 1 wherein a catalyst support is provided on the conductor (3) layer for carbon nanotubes (CNT) to be grown thereon.

10. An electrode for detecting a metabolite according to Claim 9 wherein the catalyst support is chosen from any one of silicon oxide, silicon nitride, titanium nitride, indium tin oxide or aluminium oxide.

11. An electrode for detecting a metabolite according to Claim 10 wherein nickel catalyst in liquid form is deposited on the catalyst support.

12. A method for fabricating an electrode for detecting a metabolite comprising the steps of:

(i) depositing a catalyst support layer (4) onto a substrate (2);
(ii) applying a catalyst on said catalyst support layer (4);
(iii) growing carbon nanotubes (CNT);
(iv) depositing a conductor layer which makes electrical contact and overlaps with the grown CNT;
(v) forming dam structure in a manner for containing a space over said grown CNT;
(vi) dispensing a CNT-ferrocene solgel composite mixture into said dam structure over said CNT.

13. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the catalyst support layer is depositing via screen printing through a stencil mask.

14. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the catalyst support layer (4) is comprised of a thin silicon oxide layer of about 50 nm, patterned with circular shape of 1 mm diameter or square shape of 1 mm × 1 mm with dry oxidation process on a 15.2 cm (6-inch) silicon wafer.

15. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the CNT is grown by chemical vapour deposition (CVD) process.

16. A method for fabricating an electrode for detecting a metabolite according to Claim 15 wherein the CVD process occurs under flow of acetylene and ammonia gasses at temperature in the range of 600 – 850°C and chamber pressure of 1 Torr.

17. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the dam structure is formed by epoxy paste being dispensed, including by screen-printing.

18. A method for fabricating an electrode for detecting a metabolite according to Claim 17 wherein the dam structure formed is cured at 90° – 120°C for a period from 30 – 90 minutes.

19. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the dispensed CNT-ferrocene solgel composite mixture is cured at 80° – 120°C for at least 5 minutes and allowed to cool to room temperature for at least an hour.

20. A method for fabricating an electrode for detecting a metabolite according to Claim 12 wherein the dispensed CNT-ferrocene solgel composite mixture is dried under continuous flow of nitrogen gas for 1 hour.

21. A method for fabricating a sol-gel composite for use in an electrode according

to Claim 1 comprising vigorously mixing a carbon-based electrochemical-variable transducing medium, a solgel starting material, an alkoxy cross-linker, a dispersant cross-linker and an electron transfer mediator.

22. A method for fabricating a sol-gel composite for use in an electrode according to Claim 21 comprising a carbon nanostructure material, a tetraalkyl orthosilicate, alkylalkoxysilane, a trifunctional alkoxysilane, and a metallocene.

23. A method for fabricating a CNT-ferrocene solgel composite for use in an electrode according to Claim 21 comprising the steps of:

(i) mixing CNT, ferrocene, tetraethylorthosilicate, methyltriethoxysilane and phenyltriethoxysilane in a solution of hydrochloric acid;

(ii) stirring vigourously aforesaid mixture at room temperature;

(iii) leaving said mixture in a tightly-capped vial for at least an hour.

24. A method for fabricating a CNT-ferrocene solgel composite according to Claim 23 wherein the constituents comprise by weight:

- CNT – 10-35%;
- tetraethylorthosilicate – 20-40%;
- methyltriethoxysilicon – 20-40%;
- phenyltriethoxysilicon – 20-40%; and
- ferrocene – 0.1 to 10%

which are mixed in 0.05 M to 1 M hydrochloric acid prepared with deionized water.

25. A method for fabricating a CNT-ferrocene solgel composite according to Claim 24 wherein the ratio of tetraethylorthosilicate : methyltriethoxysilane : phenyl-triethoxysilane are first mixed in equal proportions in 0.1 M hydrochloric acid.

26. A method for fabricating a CNT-ferrocene solgel composite according to Claim 25 wherein 10% (by weight) single-walled CNT is added to the mixture and 1% (by weight) ferrocene is added to the mixture.

27. A method for fabricating a CNT-ferrocene solgel composite according to

Claim 26 wherein the mixture is sonicated for about 1 minute and the sonicated composition is left to stand for about 20 hours.

28. A CNT-ferrocene solgel composite for use in an electrode according to Claim 1 wherein said solgel composite has been fabricated with a method according to any one of Claims 21 – 27.

29. A CNT-ferrocene solgel composite for use in an electrode according to Claim 28 wherein said solgel composite has distinct oxidation potentials for different metabolites which are discernible from voltametric analysis.

***

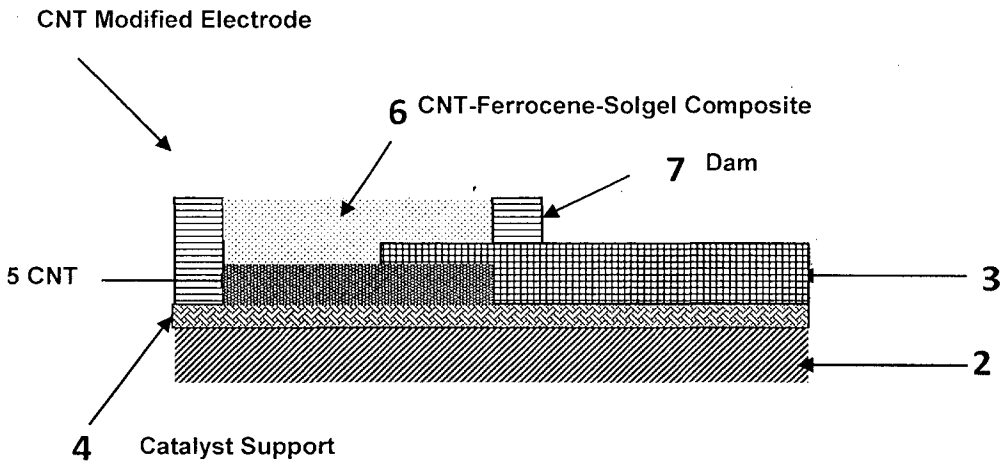

FIGURE 1

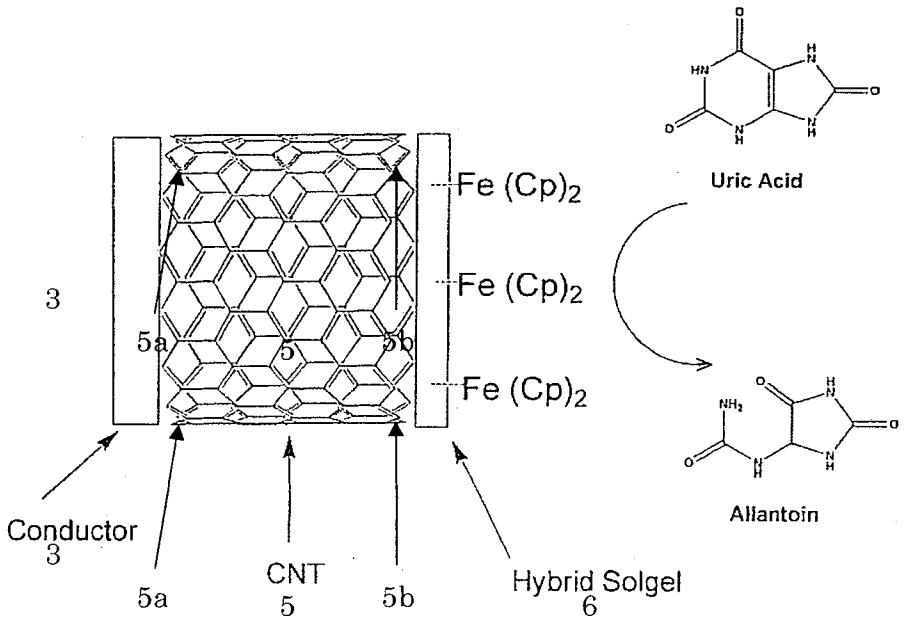

FIGURE 2

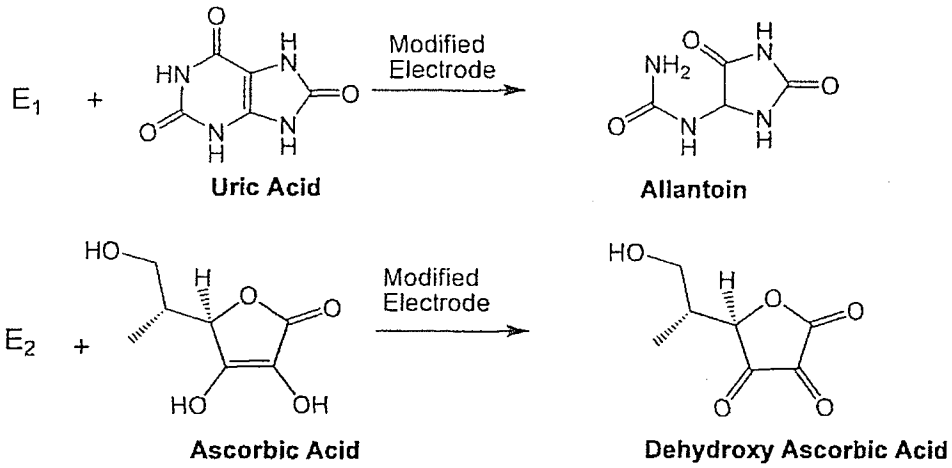

FIGURE 3

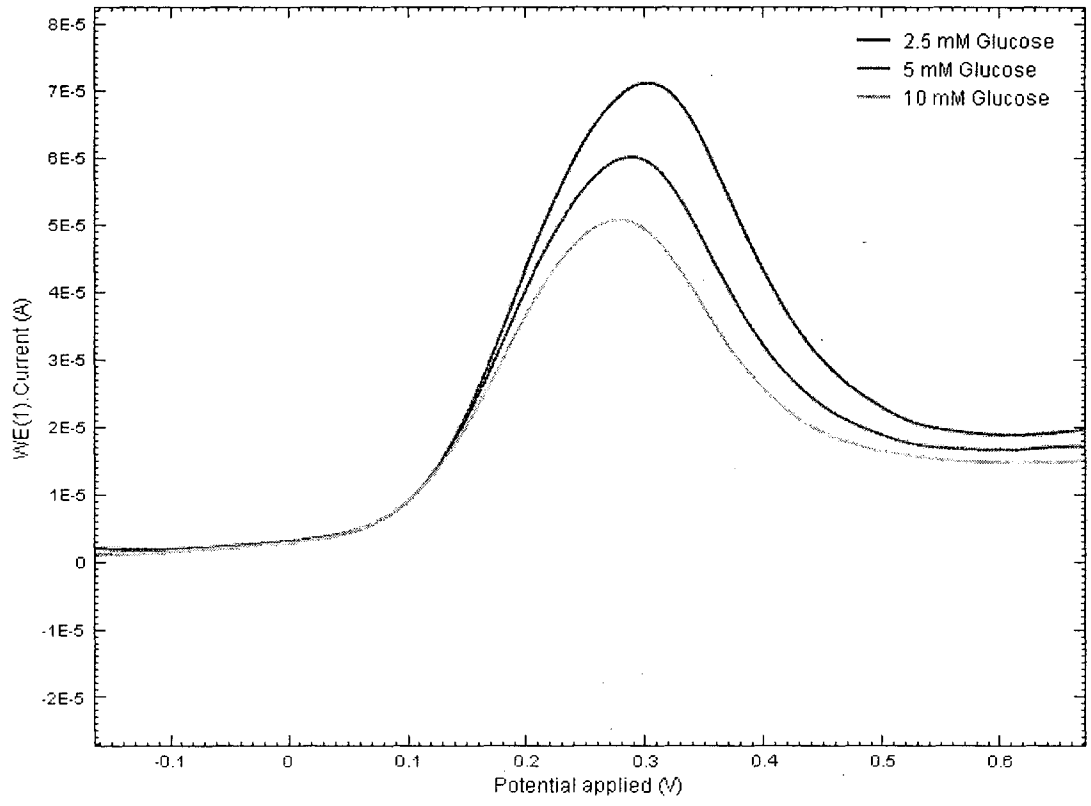

FIGURE 4

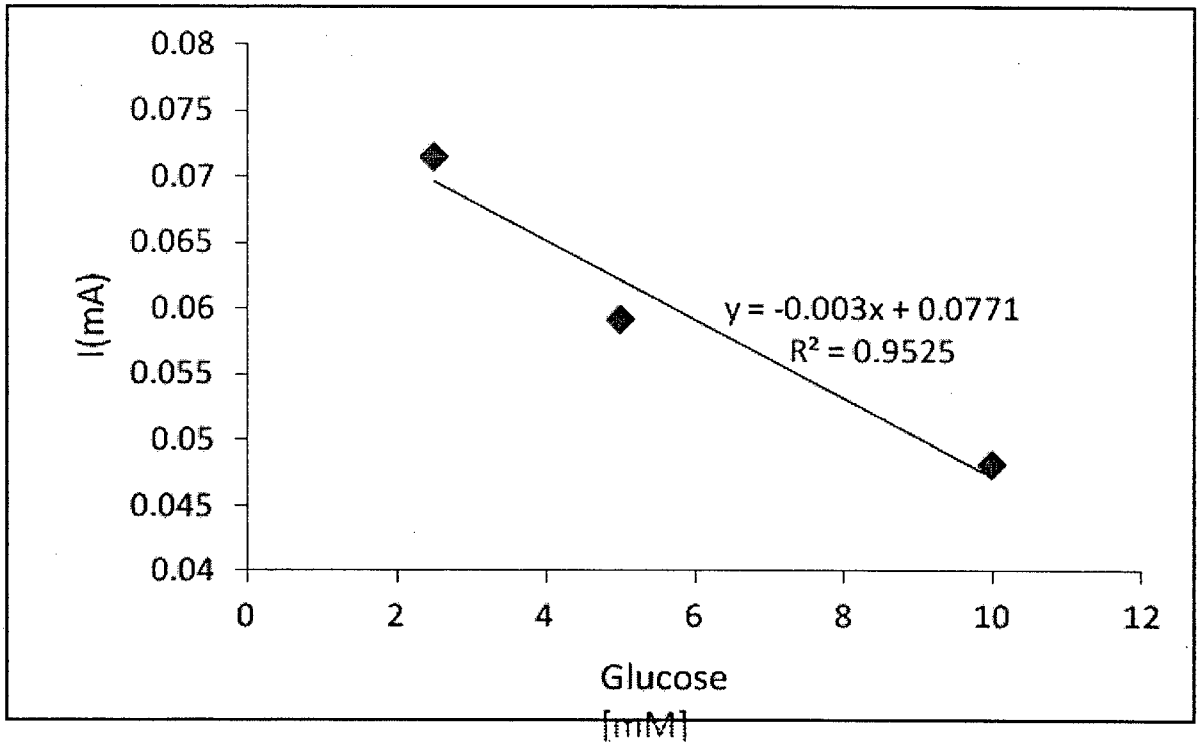
0.08
0.075
0.07
0.065
0.06
0.055
0.05
0.045
0.04
I(mA)
y = -0.003x + 0.0771
R² = 0.9525
0
2
4
6
8
10
12
Glucose
[mM]

FIGURE 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2012/000032

A. CLASSIFICATION OF SUBJECT MATTER

G01N 27/327 (OCT 2005) G01N 33/00 (OCT 2005) B82Y 5/00 (JAN 2011) B82Y 15/00 (JAN 2011) B82Y 30/00 (JAN 2011)

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI and EPODOC Keywords: G01N 27/00, G01N 33/00, B82Y; carbon, CNT; ferrocene, fecp2, metallocene; sol gel, composite, hybrid; electrode, conduct; glucose and similar terms
Google and INSPEC Keywords: carbon, CNT; sense, detect, measure, analyse, biosensor, bio assay, lab on chip, electrochemical; sol gel; electrode; ferrocene; metabolite, glucose, cholesterol, lactate, uric acid and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [ ] 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 application or patent 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 July 2012	23 July 2012

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/MY2012/000032

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/MY2012/000032**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QIU, J.-D. et al, "A nanocomposite chitosan based on ferrocene-modified silica nanoparticles and carbon nanotubes for biosensor application", Electroanalysis, 2007, vol. 19, no. 22, pages 2335-2341 Abstract, Section 1 - Introduction and Section 2 - Experimental	1-20
A	LI, J. et al, "Mediated amperometric glucose sensor modified by the sol-gel method", Sensors and Actuators B, 1997, Vol. 40, pages 135-141 Abstract	1-20
A	WANG, J., "Carbon-nanotube based electrochemical biosensors: a review", Electroanalysis, 2005, vol. 17, no. 1, pages 7-14 Abstract and Section 2.2.1	1-20
A	QIU, J.-D. et al, "Amperometric sensor based on ferrocene-modified multiwalled carbon nanotube nanocomposites as electron mediator for the determination of glucose", Analytical Biochemistry, 2009, vol. 385, pages 264-269 Whole document	1-20

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- **Claims 1-20** relate to an electrode for detecting at least a metabolite, and a method of fabricating said electrode. The features of an electrode having a conductor layer on a substrate, carbon nanotubes with a basal end disposed on the conductor layer, and a carbon nanotube-ferrocene solgel deposited on the distal end of the carbon nanotubes is specific to this group of claims.

- **Claims 21-29** relate to a method of producing a sol-gel for use in an electrode for detecting at least a metabolite, and the sol-gel itself. The feature of mixing a combination of compounds to make a sol-gel is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied a priori.