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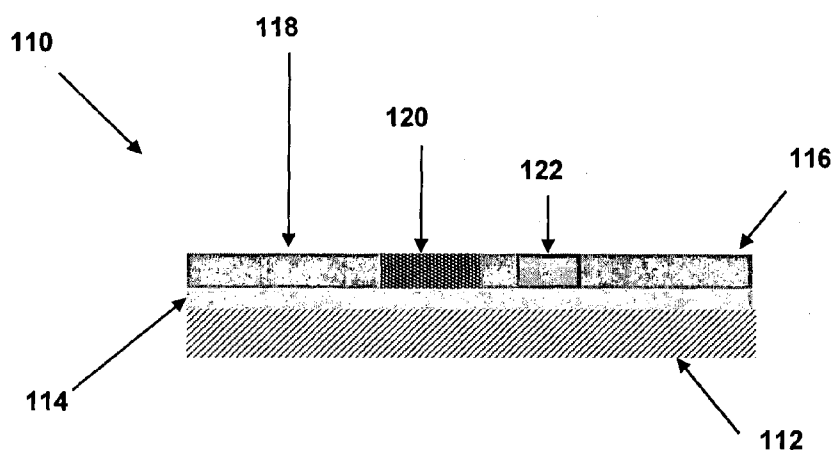


Figure 1

(57) Abstract: The present invention relates a method for producing a non-enzymatic sensor and more particularly a non-enzymatic sensor for detection of uric acid. One of the advantages of the method to present invention is to provide a simplified method for producing a non-enzymatic sensor detecting uric acid which does not require any bio-active substances and thus simplifies the production method. The method reduces the manufacturing costs and can rapidly proceed on a large scale so that the produced electrode is convenient for people to use. Another advantage of the non-enzymatic sensor for detecting uric acid can be reused for a long time.



A NON-ENZYMATIC SENSOR

FIELD OF THE INVENTION

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The present invention relates a method for producing a non-enzymatic sensor and more particularly a non-enzymatic sensor for detection of uric acid.

BACKGROUND OF THE INVENTION

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In human and many other animals, purine nucleotides undergo degradation to produce uric acid that is excreted from the body. Xanthine and hypoxanthine intermediates appear in the degradation pathways before uric acid is produced. However, in the earlier part of the pathways, inosine and guanine are produced and most of these earlier intermediates can be
15 absorbed and reused by the body. In many animals such as fish, mollusks, turtles and many animals other than primates, uric acid is further degraded to allantoin by urate oxidase. In human, other primates and birds uric acid is excreted as the final product.

Measurement of uric acid in body fluids has been widely performed to diagnose gout.
20 Enzyme-based biosensors using uricase as the active enzyme has been the preferred method to analyze uric acid in body metabolites. However, enzymatic biosensors have many disadvantages due to instability and lack of processability of enzymes. Special storage requirement is often required and denaturing of enzymes causes inaccurate sensor results. Moreover, reactive residues in enzyme such as thiol, alcohol, amine and carboxylic
25 can react during immobilization process and thus change the enzyme three-dimensional structure and reduce its activity. Storage and handling of enzyme can be quite troublesome – in order to avoid denaturing, enzyme should be stored at low temperature, and this is difficult for volume production and distribution of enzyme-based sensors.

30 The present invention overcomes these and other deficiencies of the above-mentioned drawbacks by providing a method producing a non-enzymatic sensor and more particularly a non-enzymatic sensor for detection of uric acid. The invention provides a considerable reduction of materials with even greater efficiency and economically during operation.

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SUMMARY OF THE INVENTION

The present invention provides a method for producing a non-enzymatic sensor for detecting uric acid comprising screen printing a conductor layer onto a substrate; providing a layer of graphite-carbon nanotubes – ferrocene composite onto the conductor layer via screen printing process to form a sensor; and characterizing the sensor for detecting uric acid.

In one of the embodiment, the conductor layer is selected from screen printed silver, electrodeposited silver, electroless deposited silver, platinum, gold, carbon or the like.

In yet another embodiment, the conductor layer is screen printed over metal stencil on the substrate to produce a thick film conductor electrode. The thick film is cured in temperature approximately 120°C under continuous flow of nitrogen gas for approximately 20 minutes to form a dry thickness of approximately of 100 µm.

A method of producing graphite-carbon nanotube – ferrocene composite comprising mixing a graphite powder to solvents to form graphite paste; mixing carbon nanotube flakes to solvents to form a carbon nanotube paste; diluting equivalent weight of graphite and carbon nanotube pastes in tetrahydrofuran (THF) to form graphite - carbon nanotube solution; providing ferrocene, glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer and para-xylene to the graphite carbon nanotube solution; and mixing the mixture for approximately 8 hours under nitrogen gas at ambient temperature.

In one of the embodiment, the solvents are one or combination of the following solvents; carbitol, butyl carbitol, 2-methoxy ethanol, tetrahydrofuran, 2-butanone, cyclohexanone, ethyl acetate, toluene, m-xylene, p-xylene, hexanes and acrylic acid.

In one of the embodiment, a layer of graphite-carbon nanotube – ferrocene is thick film cured at approximately 120°C under continuous flow of nitrogen gas for approximately 15 minutes to form a dry thickness of approximately 60 µm.

In yet another embodiment, a series of uric acid of calibration solutions is provided to the sensor during characterization to determine the sensitivity of the sensor on uric acid concentration.

A non-enzymatic sensor for detecting uric acid concentration comprising; a substrate; a conducting film coated on the substrate for providing an electrical contact between an electrode and a readout circuitry; an insulator being coated on the conducting film having a working electrode, a counter electrode and a reference electrode for providing a cover to conductor traces; a working electrode for allowing selective oxidation of analytes; a counter electrode to complete flow of current in a voltammetric measurement setup; and a reference electrode for providing as reference in a voltammetric measurement.

In one of the embodiment, the working electrode comprises of a ratio in weight of 0 to 95% of graphite, 0 to 95% of carbon nanotubes, 0.1 to 30% of ferrocene and 1 to 30% of organic binder.

In yet another embodiment, the binder is selected at least one or combination of Bisphenol A propoxylate diglycidyl ether, methyl methacrylate-glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer, glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer.

In one of the embodiment, the binder is cross-linked with at least one or combination of para-xylylenediamine, meta-xylylenediamine, para-phenylenediamine, 1,6-hexanediamine.

In one of the embodiment, the conducting film is selected from one or combination of screen printed silver, electrodeposited silver, electroless deposited silver, platinum, gold and carbon.

In yet another embodiment, the counter electrode is selected from one or combination of platinum, graphite, glassy carbon and pyrolytic carbon.

In one of the embodiment, the reference electrode is selected from silver-silver chloride, platinum-silver, platinum, mercury-mercury chloride.

In one of the embodiment, the conducting film is screen printed on the substrate.

In one of the embodiment, the insulator is screen printed on the conducting film.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate embodiments of the invention and, together with the description, serve to explain the principles of the invention.

Figure 1 illustrates a non-enzymatic sensor for detecting uric acid in accordance of an embodiment of the present invention.

Figure 2 illustrates a carbon composite electrode used to selectively oxidize uric acid to allantoin in a simple and reproducible voltammetric setup in accordance of an embodiment of the present invention.

Figure 3 illustrates a graph of Cyclic Voltammetry plots of uric acid, glucose, ascorbic acid and hydroquinone on carbon composite electrode in accordance of an embodiment of the present invention.

Figure 4 illustrates a graph of Cyclic Voltammetry plots of uric acid in different concentrations of uric acid (0.2 mM, 0.4mM, 0.6 and 0.8 mM) buffered by phosphate at pH 7 in accordance of an embodiment of the present invention.

Figure 5 illustrates a graph plot of oxidation current I (mA) versus concentration of glucose at 0.6 V in accordance of an embodiment of the present invention.

DETAILED DESCRIPTIONS OF THE INVENTION

The present invention will now be described in detail in connection with specific embodiments with reference to the accompanying drawings. Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense that is as "including, but not limited to". Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an

embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. Furthermore, in those instances where a convention analogous to "at least one of A, B and C," etc. is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B and C together, etc.). In those instances where a convention analogous to "at least one of A, B or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., "a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase "A or B" will be understood to include the possibilities of "A" or "B" or "A and B."

The term "electrode" as used herein is a broad term, and is to be given its ordinary and customary meaning to a person of ordinary skill in the art (and it is not to be limited to a special or customized meaning), and refers without limitation to a conductor through which electricity enters or leaves something such as a battery or a piece of electrical equipment. In one embodiment, the electrodes are the metallic portions of a sensor (e.g., electrochemically reactive surfaces) that are exposed to the extracellular milieu, for detecting the analyte. In some embodiments, the term electrode includes the conductive wires or traces that electrically connect the electrochemically reactive surface to connectors (for connecting the sensor to electronics) or to the electronics.

The term "calibration" as used herein is a broad term, and is to be given its ordinary and customary meaning to a person of ordinary skill in the art (and it is not to be limited to a special or customized meaning), and refers without limitation to the relationship and/or the

process of determining the relationship between the sensor data and corresponding reference data, which may be used to convert sensor data into meaningful values substantially equivalent to the reference. In some embodiments, namely in continuous analyte sensors, calibration may be updated or recalibrated over time if changes in the relationship between the sensor and reference data occur, for example due to changes in sensitivity, baseline, transport, metabolism, or the like.

The terms "insulative properties," "electrical insulator" and "insulator" as used herein are broad terms, and are to be given their ordinary and customary meaning to a person of ordinary skill in the art (and is not to be limited to a special or customized meaning) and refers without limitation to the tendency of materials that lack mobile charges to prevent movement of electrical charges between two points. In one exemplary embodiment, an electrically insulative material may be placed between two electrically conductive materials, to prevent movement of electricity between the two electrically conductive materials. In some embodiments, the terms refer to a sufficient amount of insulative property (e.g., of a material) to provide a necessary function (electrical insulation). The terms "insulator" and "non-conductive material" can be used interchangeably herein.

Figure 1 illustrates a non-enzymatic sensor (110) for detecting uric acid in accordance of an embodiment of the present invention. A non-enzymatic sensor (110) for detecting uric acid concentration of the present invention comprising a substrate (112), a conducting film (114) coated on the substrate (112) for providing an electrical contact between an electrode and a readout circuitry, an insulator (116) being coated on the conducting film (114) having a working electrode (120), a counter electrode (118) and a reference electrode (122) for providing a cover to conductor traces, a working electrode (120) for allowing selective oxidation of analytes, a counter electrode (118) to complete flow of current in a voltammetric measurement setup and a reference electrode (122) for providing as reference in a voltammetric measurement. The conducting film is screen printed on the substrate while the insulator is screen printed on the conducting film.

The working electrode (120) further comprises of a ratio in weight of 0 to 95% of graphite, 0 to 95% of carbon nanotubes, 0.1 to 30% of ferrocene and 1 to 30% of organic binder. The organic binder used is selected at least one or combination of Bisphenol A propoxylate didlycidyl ether, methyl methacrylate-glycidyl methacrylate-tetrahydrofurfuryl acrylate

copolymer, glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer. The binder is then cross-linked with at least one or combination of para-xylylenediamine, meta-xylylenediamine, para-phenylenediamine, 1,6-hexanediamine. The conducting film is selected from one or combination of screen printed silver, electrodeposited silver, electroless deposited silver, platinum, gold and carbon. The counter electrode is selected from one or combination of platinum, graphite, glassy carbon and pyrolytic carbon. The reference electrode is selected from silver-silver chloride, platinum-silver, platinum, mercury-mercury chloride.

Figure 2 illustrates a carbon composite electrode used to selectively oxidize uric acid to allantoin in a simple and reproducible voltammetric setup in accordance of an embodiment of the present invention. Other biomolecules, nutrients and drugs having similar oxidation potentials can be selectively analyzed using this technique because these molecules would produce distinct peaks upon voltammetric treatment. Therefore, oxidation of uric acid in the presence of ascorbic acid and glucose would produce three separate peaks as illustrated in

Figure 3.

A method for producing a non-enzymatic sensor for detecting uric acid of the present invention comprising firstly screen printing a conductor layer onto a substrate, followed by providing a layer of graphite-carbon nanotube – ferrocene composite onto the conductor layer via screen printing process to form a sensor; and characterizing the sensor for detecting uric acid.

The conductor layer is selected from screen printed silver, electrodeposited silver, electroless deposited silver, platinum, gold, carbon or the like. The conductor layer is screen printed over metal stencil on the substrate to produce a thick film conductor electrode. The thick film is cured in temperature approximately 120°C under continuous flow of nitrogen gas for approximately 20 minutes to form a dry thickness of approximately of 100 µm. A layer of graphite-carbon nanotube – ferrocene is thick film cured at approximately 120°C under continuous flow of nitrogen gas for approximately 15 minutes to form a dry thickness of approximately 60 µm. A series of uric acid of calibration solutions is provided to the sensor during characterization to determine the sensitivity of the sensor on uric acid concentrations.

A method for producing graphite-carbon nanotube – ferrocene composite comprising mixing a graphite powder to solvents to form graphite paste, mixing carbon nanotube flakes to solvents to form a carbon nanotube paste, diluting equivalent weight of graphite and carbon

nanotube pastes in tetrahydrofuran (THF) to form graphite - carbon nanotube solution, providing ferrocene, glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer and para-xylylene to the graphite carbon nanotube solution; and mixing the mixture for approximately 8 hours under nitrogen gas at ambient temperature. The solvents used in this method are one or combination of the following solvents; carbitol, butyl carbitol, 2-methoxy ethanol, tetrahydrofuran, 2-butanone, cyclohexanone, ethyl acetate, toluene, m-xylene, p-xylene, hexanes and acrylic acid.

The present invention is further described but not limited to the following experiments.

Experiments

Preparation of Screen Printed Silver Electrode

Silver paste was screen printed over metal stencil on 0.8mm thick FR4 substrate to produce circular shaped thick film silver electrodes with 2-3mm diameters. The thick film cured in the oven at 120 °C under continuous flow of nitrogen for 20 minutes to afford a dry thickness of 100µm. Solder mask paste was printed over metal stencil to cover the whole sensor surface and to open only the electrode windows and electrical contacts.

Preparation of Graphite-Carbon Nanotube-Ferrocene Composite

Graphite powder (less than 20 µm) was mixed with butyl carbitol to form a thick paste. Similarly carbon nanotubes flakes (less than 20 µm) were formed into thick paste with butyl carbitol. Graphite and CNT pastes, each weighed 1g, were diluted with 20mL tetrahydrofuran (THF). Ferrocene (0.1g), glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer (0.2g) and para-xylylene were added to the graphite-CNT solution in THF. The resulting mixture was magnetically stirred for 8 hours under nitrogen ambient. The excess THF was evaporated to afford thick composite paste.

Preparation of Uric Acid Sensor

Graphite-CNT-ferrocene composite paste was screen printed over metal stencil onto the silver layer in the working electrode window. The composite thick film cured in the oven at 120 °C under continuous flow of nitrogen for 15 minutes to afford a dry thickness of 60µm.

Characterization of Uric Acid Sensor

The uric acid sensor was characterized with Metrohm Autolab PGSTAT MODEL 128N system. The calibration solutions (0.2 mM, 0.4mM, 0.6mM and 10 mM uric acid were buffered with phosphate at pH7. Cyclic voltammetry and linear sweep voltammetry were conducted for the above four solutions of uric acid 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 0.6 V were observed as shown in **Figure 4**.

Table 1 below shows oxidation peaks at voltage of about 0.6V for four uric acid concentrations.

[Uric Acid](mM)	I(mA)
0.2	0.0206
0.4	0.0405
0.6	0.06
0.8	0.0778
M	0.0955
C	0.002
r ²	0.9994

Table 1: Oxidation peak currents (I) of three concentrations of glucose

Figure 5 illustrates a graph plot of oxidation current I (mA) versus concentration of glucose at 0.6 V in accordance of an embodiment of the present invention. This result showed that there a strong relationship between the oxidation current and concentration of glucose when the uric acid sensor was characterized.

One of the advantages of the method to present invention is to provide a simplified method for producing a non-enzymatic sensor detecting uric acid which does not require any bio-

active substances and thus simplifies the production method. The method reduces the manufacturing costs and can rapidly proceed on a large scale so that the produced electrode is convenient for people to use. Another advantage of the non-enzymatic sensor for detecting uric acid can be reused for a long time.

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The foregoing embodiment and advantages are merely exemplary and are not to be construed as limiting the present invention. The description of the embodiments of the present invention is intended to be illustrative and not to limit the scope of the claims and many alternatives, modifications and variations will be apparent to those skilled in the art.

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CLAIMS

1. A method for producing a non-enzymatic sensor for detecting uric acid comprising
5 screen printing a conductor layer onto a substrate;
providing a layer of graphite-carbon nanotube – ferrocene composite onto the conductor
layer via screen printing process to form a sensor; and
characterizing the sensor for detecting uric acid.
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2. The method as claimed in Claim 1 wherein the conductor layer is selected from screen
printed silver, electrodeposited silver, electroless deposited silver, platinum, gold, carbon
or the like.
- 15 3. The method as claimed in Claim 1 wherein the conductor layer is screen printed over
metal stencil on the substrate to produce a thick film conductor electrode.
4. The method as claimed in Claim 3 wherein the thick film is cured in temperature
approximately 120°C under continuous flow of nitrogen gas for approximately 20 minutes
20 to form a dry thickness of approximately of 100 µm.
5. The method as claimed in Claim 1 wherein a method of producing graphite-carbon
nanotube – ferrocene composite comprising
25 Mixing a graphite powder to solvents to form graphite paste;
Mixing carbon nanotube flakes to solvents to form a carbon nanotube paste;
Diluting equivalent weight of graphite and carbon nanotube pastes in tetrahydrofuran
(THF) to form graphite - carbon nanotube solution;
Providing ferrocene, glycidyl methacrylate-tetrahydrofuryl acrylate copolymer and para-
30 xylylene to the graphite carbon nanotube solution; and
Mixing the mixture for approximately 8 hours under nitrogen gas at ambient temperature.
6. The method as claimed in Claim 5 wherein the solvents are one or combination of the
following solvents; carbitol, butyl carbitol, 2-methoxy ethanol, tetrahydrofuran, 2-

butanone, cyclohexanone, ethyl acetate, toluene, m-xylene, p-xylene, hexanes and acrylic acid.

7. The method as claimed in Claim 1 wherein a layer of graphite-carbon nanotube – ferrocene is thick film cured at approximately 120°C under continuous flow of nitrogen gas for approximately 15 minutes to form a dry thickness of approximately 60 µm.
8. The method as claimed in Claim 1 wherein a series of uric acid of calibration solutions is provided to the sensor during characterization to determine the sensitivity of the sensor on uric acid concentration.
9. A non-enzymatic sensor for detecting uric acid concentration comprising;
 - a substrate;
 - a conducting film coated on the substrate for providing an electrical contact between an electrode and a readout circuitry;
 - an insulator being coated on the conducting film having a working electrode, a counter electrode and a reference electrode for providing a cover to conductor traces;
 - a working electrode for allowing selective oxidation of analytes;
 - a counter electrode to complete flow of current in a voltammetric measurement setup;
 - and
 - a reference electrode for providing as reference in a voltammetric measurement.
10. The non-enzymatic sensor as claimed in Claim 9 wherein the working electrode comprises of a ratio in weight of 0 to 95% of graphite, 0 to 95% of carbon nanotubes, 0.1 to 30% of ferrocene and 1 to 30% of organic binder.
11. The non-enzymatic sensor as claimed in Claim 10 wherein the binder is selected at least one or combination of Bisphenol A propoxylate diglycidyl ether, methyl methacrylate-glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer, glycidyl methacrylate-tetrahydrofurfuryl acrylate copolymer.
12. The non-enzymatic sensor as claimed in Claim 10 wherein the binder is cross-linked with at least one or combination of para-xylylenediamine, meta-xylylenediamine, para-phenylenediamine, 1,6-hexanediamine.

13. The non-enzymatic sensor as claimed in Claim 9 wherein the conducting film is selected from one or combination of screen printed silver, electrodeposited silver, electroless deposited silver, platinum, gold and carbon.

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14. The non-enzymatic sensor as claimed in Claim 9 wherein the counter electrode is selected from one or combination of platinum, graphite, glassy carbon and pyrolytic carbon.

10 15. The non-enzymatic sensor as claimed in Claim 9 wherein the reference electrode is selected from silver-silver chloride, platinum-silver, platinum, mercury-mercury chloride.

16. The non-enzymatic sensor as claimed in Claim 9 wherein the conducting film is screen printed on the substrate.

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17. The non-enzymatic sensor as claimed in Claim 9 wherein the insulator is screen printed on the conducting film.

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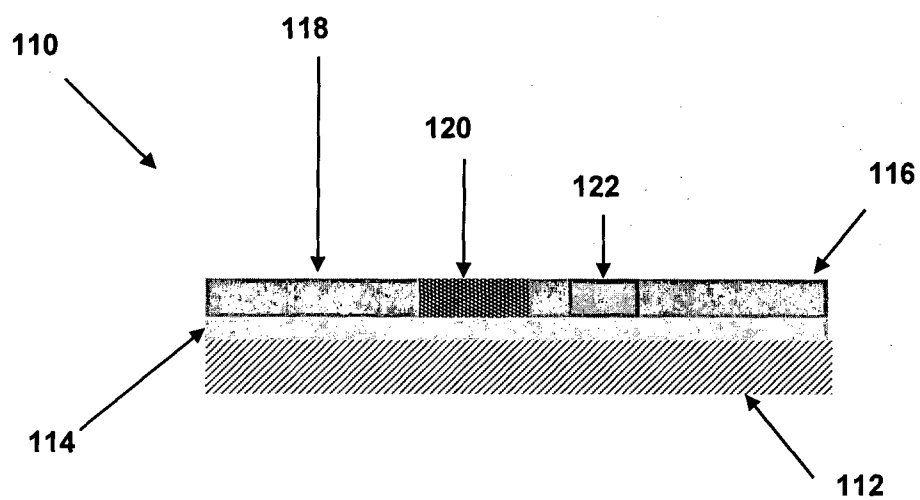
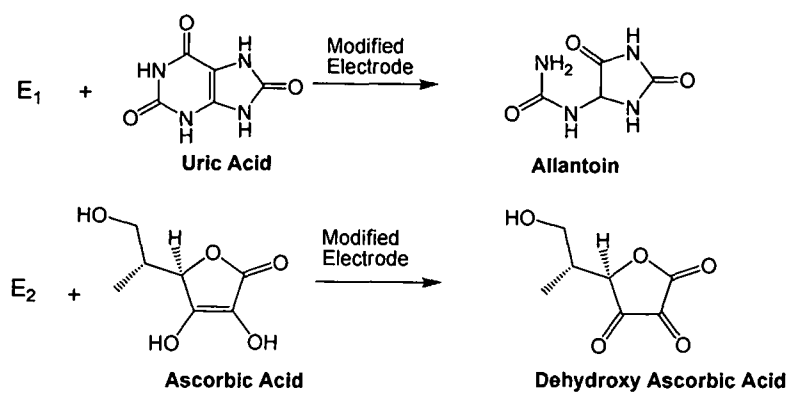
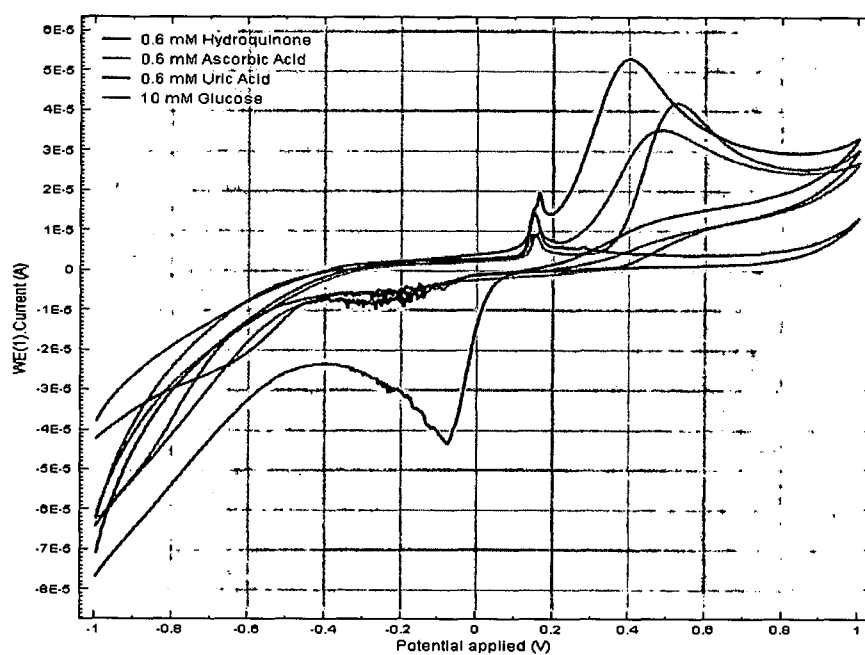
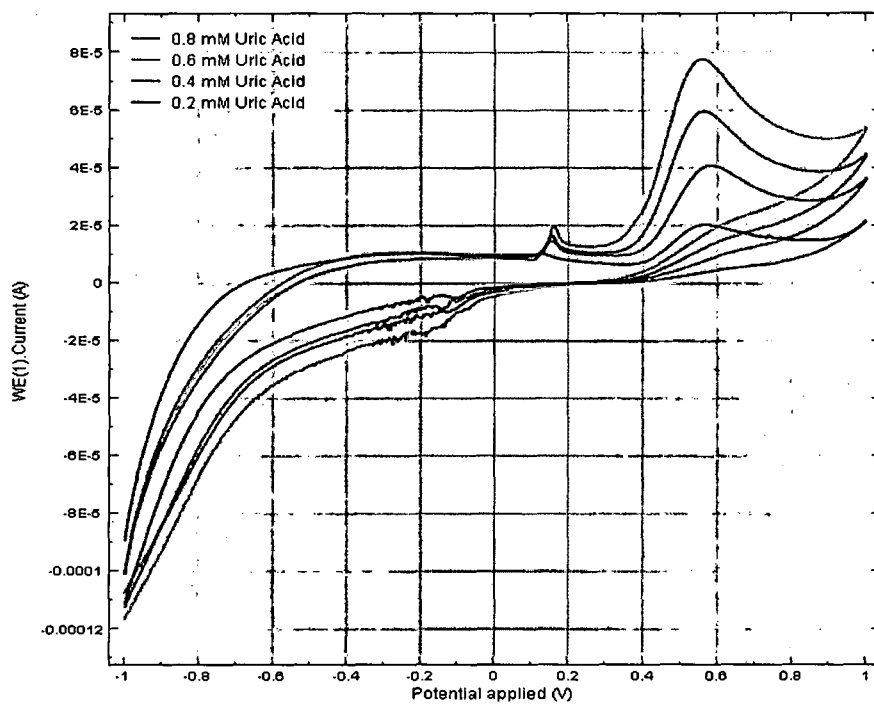
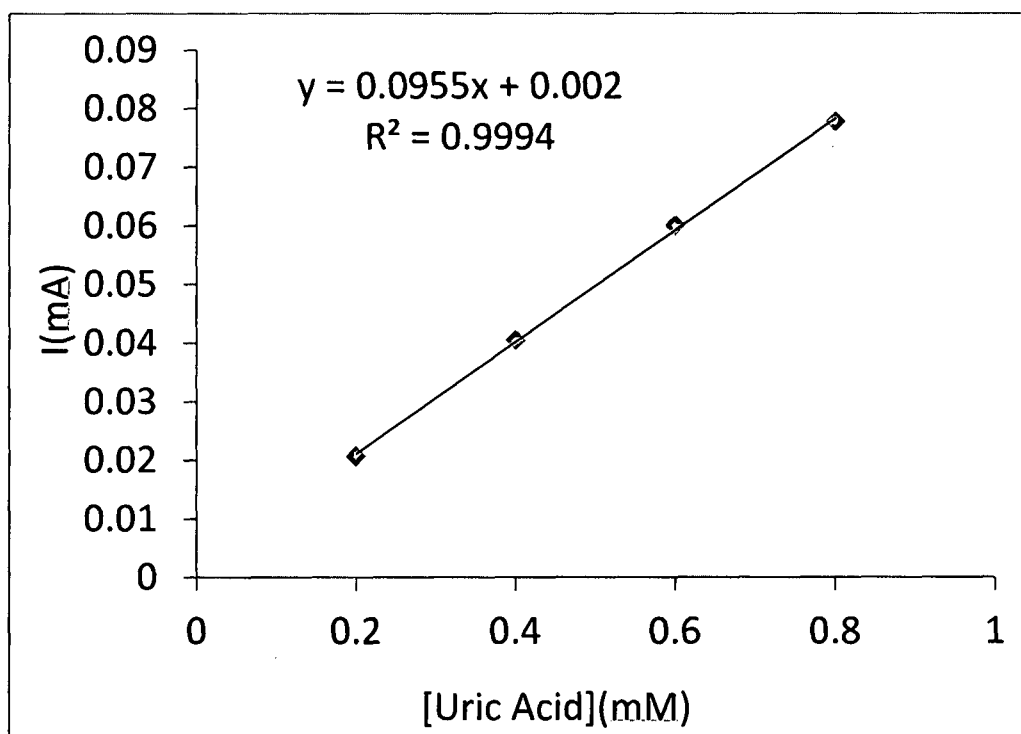


Figure 1

**Figure 2****Figure 3**

**Figure 4****Figure 5**