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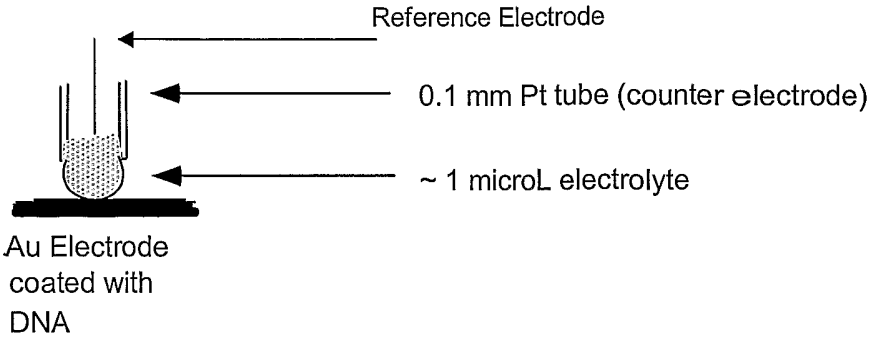
(54) Title: SMALL VOLUME ELECTROCHEMICAL ANALYSIS SYSTEM



(57) Abstract: In various aspects, the invention provides an electrochemical analysis system comprising a fluid-dispensing counter electrode positionable to dispense an electrolyte onto a coated working electrode, wherein electrical energy applied to the system induces a detectable signal that is indicative of a property of the coating on the working electrode.

WO 2005/088291 A1

SMALL VOLUME ELECTROCHEMICAL ANALYSIS SYSTEM

FIELD OF THE INVENTION

[0001] The invention is in the field of electrochemical cells, including cells that may be used to assay an electrochemical characteristic of a polymeric coating, such as DNA, on a working electrode.

BACKGROUND OF THE INVENTION

[0002] A wide variety of systems have been proposed for carrying out electrochemical analysis of molecular substrates. Alternative systems are for example disclosed in the following US patents, which are hereby incorporated herein by reference: 673,533; 6,618,934; 6,592,745; 6,591,125; 6,461,496; 6,458,600; 6,338,790; 6,306,584; 6,299,757; 6,207,369; 6,140,045; 6,090,545; 6,078,490; 6,066,448; 6,010,613; 5,622,872; 5,571,568; 5,491,097.

SUMMARY OF THE INVENTION

[0003] In various aspects, the invention provides an electrochemical analysis system comprising a fluid-dispensing counter electrode positionable to dispense an electrolyte onto a coated working electrode, wherein electrical energy applied to the system induces a detectable signal that is indicative of a property of the coating on the working electrode. The counter electrode may for example be tubular, adapted to dispense an electrolyte from the lumin of the tube. The working electrode may for example be coated with a molecular substrate, such as an organic compound or a polymer. In some embodiments the coating on the working electrode may be a biological molecule or polymer, such as a nucleic acid. In some embodiments, the systems of the invention may be used to measure an electrical property of the coating on the working electrode, such as systems for carrying out impedance measurements or chronoamperometry. In alternative embodiments, systems of the invention may be used to detect the interaction of the coating on the working electrode with a ligand, such as a ligand introduced into a sample chamber for binding to the coating on the working electrode or a ligand dispensed onto to the working electrode by the fluid-dispensing counter electrode.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] Figure 1. Impedance spectrum of Pt-tube electrode (Counter electrode) positioned above a flat gold electrode (Working electrode) covered in a monolayer of duplex DNA (20-mer) attached via Au-S linkage. The Pt-tube has an outer diameter of 400 µm and an inner diameter of 100 µm. Supporting electrolyte: 20 mM Tris-ClO_4 + 20 mM $NaClO_4$ + 4 mM $Fe(CN)_6^{4-/3-}$. Frequency range: 100 kHz – 0.1 Hz (5 points per decade) with a 5 mV sinusoidal excitation signal applied on top of a 0 mV applied DC potential. Reference lead was connected directly to the Counter (Pt-tube) electrode.

[0005] Figure 2. Cyclic voltammagram of a clean, flat Au electrode (Working) positioned below the Pt-tube electrode (Counter electrode) with a Teflon coated (9 µm)-Pt wire (50 µm diameter) inserted through the Pt-tube to contact the measurement solution as a quasi-reference electrode. The Pt-tube has an outer diameter of 400 µm and an inner diameter of 100 µm. Supporting electrode: 20 mM Tris-ClO4 + 20 mM NaClO4 + 4 mM Fe(CN)64-/3-. Electrochemical parameters: A triangular wave form was applied from -0.5 to +0.5 (vs. Pt quasi reference) with a gradient of 20 mV·s-1.

[0006] Figure 3A is a schematic illustration showing a platinum tube counter electrode of the invention making electrical contact with a gold electrode coated with DNA, the electrical contact being made through an electrolyte solution extruded from the tip of the tube electrode onto the surface of the coated gold working electrode.

[0007] Figure 3B is a schematic illustration of a sample chamber of the invention having eight gold working electrode disks therein. The working electrode disks may be coated, for example with a layer of one or more nucleic acids. A sample may be introduced into the sample chamber, such as a nucleic acid capable of hybridizing to one or more of the nucleic acid coatings on the working electrodes. The sample may be introduced into the sample chamber by way of an input port, and drained by way of an output port, and washing steps may similarly be performed, for example to vary the stringency of hybridization to a nucleic acid layer on the working

electrodes. The working electrodes may then be interrogated by tubular counter electrodes of the invention to measure an electrical property of the coating on the working electrode. The portion of the working electrode that is addressed by the counter electrode will be determined by the area of contact between the working electrode surface and the electrolyte extruded from the tubular counter electrode of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0008] In various aspects, the invention provides an electrochemical analysis system comprising a fluid-dispensing counter electrode, such as a Pt tube electrode. The counter electrode of the invention may be adapted to be positionable to dispense an electrolyte, such as an aqueous electrolyte. The electrolyte may be dispensed onto a coated working electrode, such as an electrode coated with a polymer or organic compound, such as DNA. The system may be adapted so that the electrical contact between the electrolyte dispensed from the counter electrode and the working electrode forms an electrochemical cell. Electrical components may be provided so that electrical energy may be applied to the cell in the system so as to induce a detectable signal that is indicative of a property of the coating on the working electrode.

[0009] In some embodiments, a plurality of working electrode binding domains may for example be provided, able to specifically bind one or more analytes of interest in a sample. The binding domains may for example be prepared as patterned, multi-array multi-specific working electrode surfaces on a support.

[0010] In one aspect, the invention provides an electrochemical system for measuring a detectable signal, such as electrochemiluminescence or impedance, in a sample, for example in a sample on a working electrode in an electrochemical cell of the invention. In such systems, a potential may be applied between a coated working electrode and an electrolyte-dispensing counter electode, for example by voltage control means, to induce the detectable signal, such as electrochemiluminescence or impedance, which is indicative of a property of the coating on the working electrode. A detector, such as impedance measurement

means or a photon detector means for detecting electrochemiluminescence, may be employed to measure the detectable characteristic of the coating.

[0011] An embodiment of the electrochemical system of the invention may for example be constructed as follows:

[0012] 1. A Platinum tube may be attached to a teflon tube at one end.

[0013] 2. If required, a teflon coated reference electrode (such as a Palladium electrode) may be threaded through the Pt tube and the teflon tube.

[0014] 3. The teflon tube may be attached to a syringe or other microfluidics device, for example leaving the reference electrode on the outside (other geometries are possible. e.g. attaching a teflon tube with a T-junction to the Pt tube; one end of the T may be attached to the syringe while the other provides an outlet for the reference electrode).

[0015] 4. The above assembly may be fixed in a micropositioner.

[0016] 5. The working electrode and the Pt tube which acts as the counter electrode may be attached to a potentiostat, for example with electrical clamps. If applicable, the reference electrode may also be attached to the potentiostat.

[0017] 6. The Pt tube may be precisely positioned at a fixed height above the working electrode. The working electrode may be pretreated with a probe, such as a protein or nucleic acid, to provide a coated working electrode. The Pt tube may be positioned over an area of relatively uniformly-coated working electrode, in some embodiments avoiding positions over any uncoated portions working electrode, such as electrical leads to the working electrode. The geometry of the working electrode may vary, and may for example be flat.

[0018] 7. A small volume of electrolyte may be dispensed from the Pt tube to contact the working electrode. The volume required to form a desired region of

contact may be determined by the height of the Pt tube above the working electrode, and by the internal diameter of the Pt tube.

[0019] 8. Electrochemical measurements may then be performed to determine an electrochemical characteristic of the coating on the working electrode.

[0020] 9. In some embodiments the Pt tube may also be used to deliver a sample containing a ligand for binding or interaction with the coating on the working electrode. The fluid-dispensing counter electrode may also be used to dispense other fluids, such as washing buffers to clean the working electrode before performing the electrochemical measurements.

[0021] In some embodiments, the surface area of the working electrode addressed by the electrochemical detection system of the invention may be determined by the diameter of the tubular fluid-dispensing counter electrode, such as the illustrated a Pt-tube electrode. In such embodiments, the effect of variations in the coating of the working electrode may be minimized by localizing the area of analysis to a uniformly coated area.

[0022] A reference electrode (such as a teflon-coated Pd reference electrode) may be inserted into a tubular fluid-dispensing counter electrode, so that the system of the invention may be used for alternative forms of electrochemical analysis, such as chronoamperometric measurements, cyclic voltammetry or direct current measurements.

[0023] The Figures show data from embodiments of the invention in which a 0.1 mm internal diameter Pt tube counter electrode was used to give reproducible impedance measurements of a DNA-coated gold surface. Alternative configurations of counter electrode, such as tubes of alternative cross sectional configurations or sizes, may be used to facilitate analysis in alternative embodiments, for example with alternative volumes of electrolyte.

[0024] Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Any priority document(s) and all publications, including but not limited to patents and patent applications, cited in this specification are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

CLAIMS

1. An electrochemical analysis system comprising a fluid-dispensing counter electrode positionable to dispense an electrolyte onto a coated working electrode to form an electrochemical cell, wherein electrical energy applied to the cell induces a detectable signal that is indicative of a property of the coating on the working electrode.

2. An electrochemical analysis system comprising: a counter electrode having means for dispensing fluid, the counter electrode being positionable to dispense an electrolyte onto a coated working electrode to form an electrochemical cell; and means for applying electrical energy to the cell to induce a detectable signal that is indicative of a property of the coating on the working electrode.

3. The electrochemical analysis system of claim 1 or 2, wherein a plurality of coated working electrodes are arranged in an array, and the counter electrode is positionable to successively interrogate the array of working electrodes.

4. The electrochemical analysis system of claim 1, 2 or 3, wherein the working electrode is housed in a sample chamber, and the sample chamber is adapted to permit the working electrode to be exposed to a sample solution for binding of a sample to the working electrode, so that the detectable signal is indicative of binding of the sample to the working electrode.

5. The electrochemical analysis system of claim 4 , wherein the sample chamber has a port for introducing the sample solution.

6. The electrochemical analysis system of any one of claims 1 through 5, wherein the coating is a nucleic acid.

7. The electrochemical analysis system of any one of claims 1 through 5, wherein the coating is a protein.

8. A method of determining a property of a coating on a coated working electrode, comprising positioning a fluid-dispensing counter electrode adjacent to the coated working electrode; dispensing an electrolyte onto the coated working electrode from the counter electrode, to form an electrochemical cell; and, applying electrical energy to the cell to induce a detectable signal that is indicative of a property of the coating on the working electrode.

9. The method of claim 8, wherein a plurality of coated working electrodes are arranged in an array, and the counter electrode is positionable to successively interrogate the array of working electrodes.

10. The method of claim 8 or 9, wherein the working electrode is housed in a sample chamber, and the sample chamber is adapted to permit the working electrode to be exposed to a sample solution for binding of a sample to the working electrode, so that the detectable signal is indicative of binding of the sample to the working electrode.

11. The method of claim 10, wherein the sample chamber has a port for introducing the sample solution.

12. The method of any one of claims 8 through 11, wherein the coating is a nucleic acid.

13. The method of any one of claims 8 through 11, wherein the coating is a protein.

Figure 1

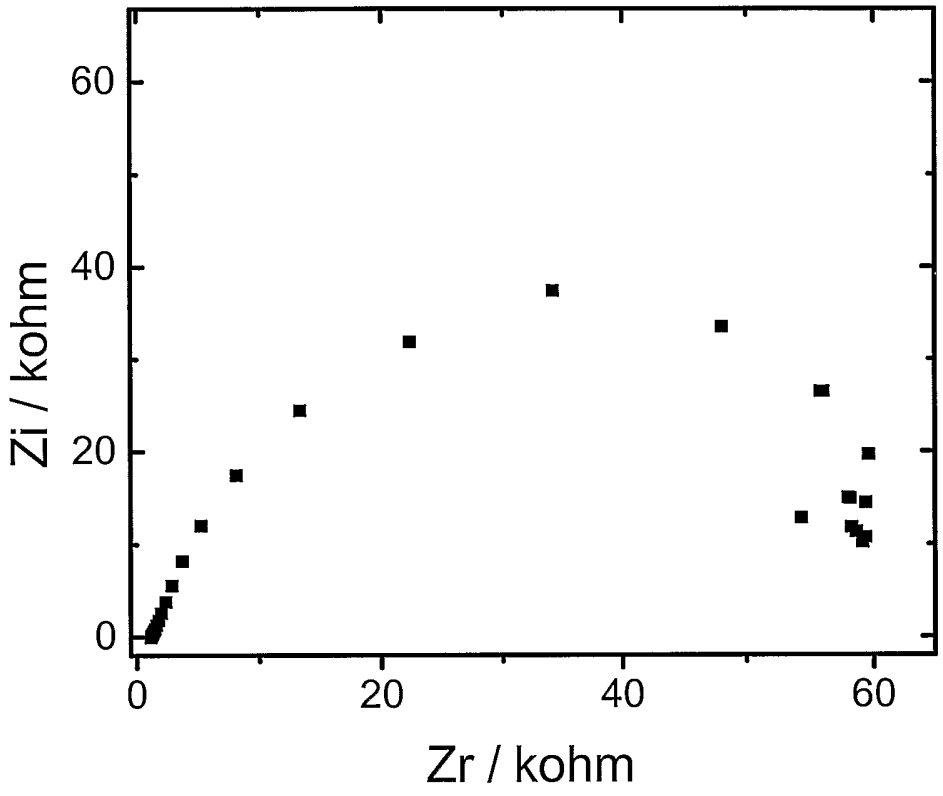


Figure 2

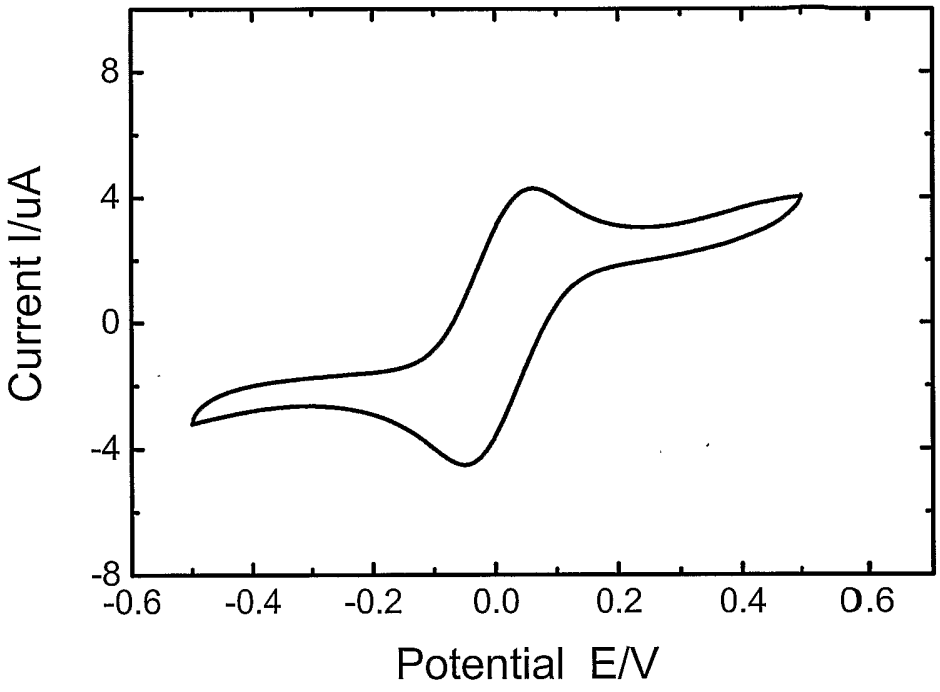
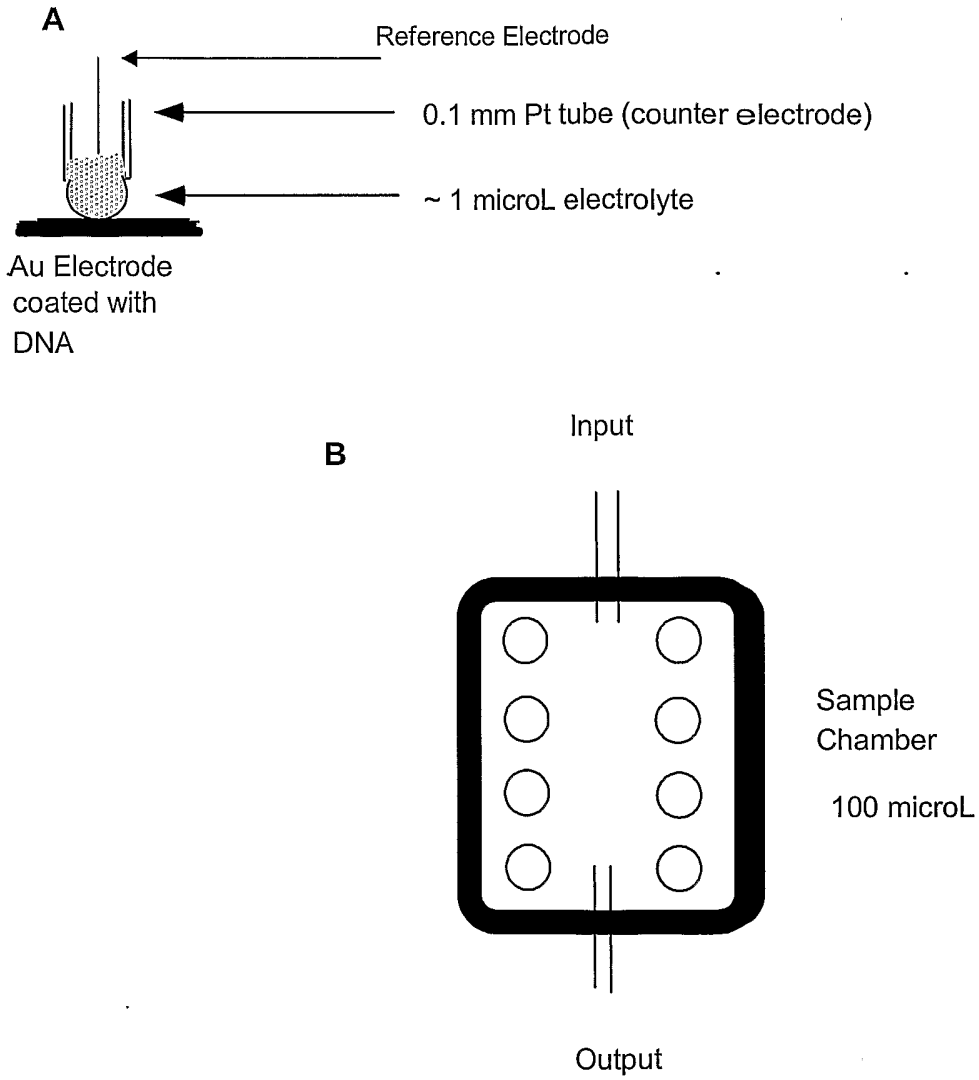


Figure 3


A
Reference Electrode
0.1 mm Pt tube (counter electrode)
~ 1 microL electrolyte
Au Electrode coated with DNA
B
Input
Sample Chamber
100 microL
Output

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000390

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7): G01N 27/42, G01N 27/327

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): G01N (using keywords)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Delphion, Esp@cenet, IEEE, United States Patent Database (USPTO).
Keywords: cell, coated, counter, electrical, electrochemical, electrode, electrolyte, impedance, nucleic acid, protein, signal, working.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6576101 B1 (THERASENSE INC.) 10 June 2003 col. 1, line 46 - col. 2, line 30 col. 6, line 42 - col. 7, line 6 col. 10, lines 1-33 col. 12, lines 21-45 col. 14, lines 21-29 col. 17, lines 40-42 col. 19, line 61 - col. 20, line 1 col. 23, lines 58-62 abstract	1-13
Y	US 6461496 B1 (THERASENSE INC.) 8 October 2002 col. 2, lines 2-40 col. 7, lines 57-67 col. 26, lines 54-62 col. 50, lines 54-65 claim 11 abstract	1-13

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2005 (07-06-2005)	7 July 2005 (07-07-2005)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Tung Nguyen (819) 956-3859

INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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