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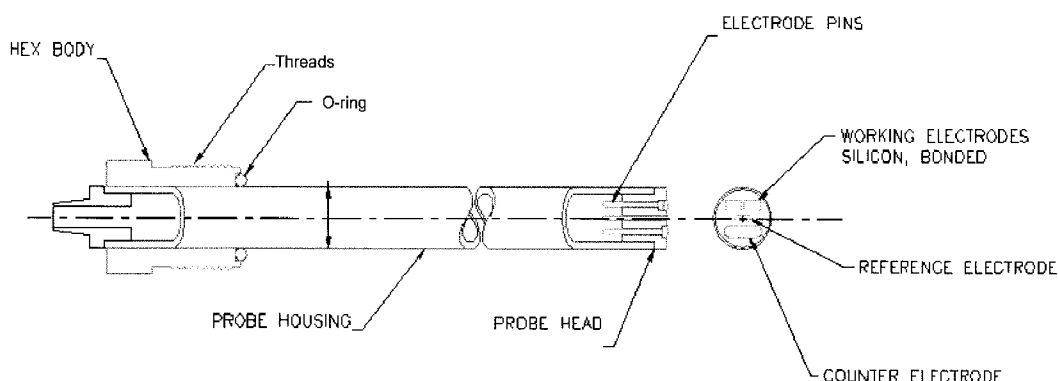
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Figure 3



(57) Abstract: Described herein are substrates, sensors and systems related to measuring the concentration of an analyte such as hydrogen ion in a sample. Redox active moieties whose reduction and/or oxidation potentials are sensitive to the presence of an analyte are immobilized onto a semiconductor surface. Immobilized redox active moieties whose reduction and/or oxidation potential are insensitive to the analyte can be used for reference. Voltammetric measurements made using such modified semiconductor surfaces can accurately determine the presence and/or concentrations of analytes in a sample of interest. The semiconductor electrochemical sensors of the invention are robust and can be made so as not to require calibration or re-calibration.

SEMICONDUCTOR ELECTROCHEMICAL SENSORS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Applications No. 60/942,646, filed June 7, 2007, 60/942,644, filed June 7, 2007, 60/942,641, filed June 7, 2007, and 60/949,805, filed July 13, 2007
5 all of which application are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] The measurement of analyte concentration, in particular, hydrogen ion concentration or pH is important in a number of research, industrial, and manufacturing processes. For instance, the measurement of pH is routinely practiced in food and beverage, biofuel, biopharmaceuticals, as well as in
10 the treatment of water and waste.

[0003] Many conventional pH sensors use a potentiometric approach which involves the use of glass electrode to measure pH. The potentiometric approach suffers from several drawbacks. One limitation of potentiometric sensors is the need for constant calibration. Potentiometric pH electrodes, like batteries, tend to run down with time and use. As the potentiometric electrode ages, its glass membrane tends to
15 change in resistance, which in turn will alter the electrode potential. For this reason, the glass electrodes require calibration on a regular basis. The need for constant recalibration to provide an accurate pH output significantly impedes industrial applications especially where constant in-line pH measurements are required. Recalibration is particularly cumbersome in a biotech environment where pH measurement is conducted in medium containing biological materials. Another significant drawback of conventional
20 pH sensors is that the glass electrodes have internal solutions, which in some cases can leak out into the solution being measured. The glass electrodes can also become fouled by species in the measuring solution, e.g. proteins, causing the glass electrode to foul. ISFET devices have been developed which use a field effect transistor structure on a silicon surface to measure pH (Bergveld Em et al., IEEE Sensor Conference, Toronto, October, 2003, 1-26). These devices also have limitations. Thus, there remains a
25 considerable need for reliable and consistent analyte sensors, and in particular, pH sensors.

INCORPORATION BY REFERENCE

[0004] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and
30 individually indicated to be incorporated by reference.

SUMMARY OF THE INVENTION

[0005] One aspect of the invention is a sensor for detecting the presence of an analyte comprising: a semiconductor electrode having a surface having immobilized thereon a redox-active moiety, wherein the
35 redox-active moiety exhibits an oxidation potential and/or a reduction potential that is sensitive to the

presence of said analyte. In some embodiments the analyte is hydrogen ion and the redox-active moiety is sensitive to hydrogen ion concentration. In some embodiments the sensor comprises a plurality of redox-active moieties, wherein at least one of the redox-active moieties is sensitive to the presence of an analyte, and at least one other redox-active moiety is substantially insensitive to the presence of said analyte.

[0006] In some embodiments the analyte is hydrogen ion, and the moiety that is substantially insensitive to the presence of hydrogen ion has a substituent selected from the group consisting of ferrocene, polyvinylferrocene, viologen, polyviologen, and polythiophene. In some embodiments the moiety that is substantially insensitive to the presence of hydrogen ion is ferrocene or a derivative of ferrocene.

[0007] In some embodiments the analyte is hydrogen, and the redox-active moiety that is sensitive to the presence of the hydrogen ion comprises a substituent selected from the group consisting of anthracenes, quinones, anthroquinones, phenanthroquinones, phenylene diamines, catechols, phenothiazinium dyes, monoquaternized N-alkyl-4,4'-bipyridinium, RuOx, and Ni(OH)₂. In some embodiments the redox-active moiety that is sensitive to the presence of hydrogen ion comprises a substituent comprising anthracene. In some embodiments the redox-active moiety that is sensitive to the presence of hydrogen ion comprises a substituent comprising an anthraquinone or a phenanthraquinone.

[0008] In some embodiments the redox-active moiety that is sensitive to the presence of an analyte is sensitive to the concentration of the analyte. In some embodiments the oxidation potential and/or reduction potential of the redox-active moiety is sensitive to the concentration of the analyte in a range from 10⁻³ M to 10⁻¹⁰ M. In some embodiments oxidation potential and/or reduction potential of the redox-active moiety is sensitive to the concentration of the analyte in a range from 10⁻¹ M to 10⁻¹⁴ M. In some embodiments the sensor detects hydrogen ion concentration from pH3 to pH10. In some embodiments the sensor detects hydrogen ion concentration from pH1 to pH14.

[0009] In some embodiments the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.1 pH unit. In some embodiments the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.01 pH units.

[0010] In some embodiments the redox-active moiety is covalently bound to the surface of the electrode. In some embodiments the redox-active moiety is bound to a polymer that is immobilized onto the surface of the electrode.

[0011] In some embodiments the semiconductor electrode is doped. In some embodiments the semiconductor electrode is P-doped. In some embodiments the semiconductor electrode comprises a silicon electrode doped with boron. In some embodiments the semiconductor electrode is N-doped. In some embodiments the semiconductor electrode comprises a silicon electrode is doped with phosphorous.

[0012] In some embodiments the semiconductor electrode comprises a monolithic piece of silicon.

[0013] In some embodiments the semiconductor electrode comprises a composite electrode, said composite electrode comprising semiconductor particles in a matrix. In some embodiments the semiconductor electrode comprises a composite electrode bound to a conductive substrate. In some

embodiments the semiconductor particles are present in an amount such that a conductive path is created across the composite electrode.

[0014] In some embodiments the electrode comprises silicon. In some embodiments the electrode comprises unpolished silicon. In some embodiments the electrode comprises polished silicon.

5 [0015] In some embodiments the semiconductor electrode has a resistivity within the range of 0.01 to 1000 Ω -cm. In some embodiments the semiconductor electrode has a resistivity within the range of 1 to 100 Ω -cm.

[0016] In some embodiments the sensor is capable of measuring analyte concentration without calibration with an external standard. In some embodiments the sensor remains sensitive to the analyte
10 without calibration after a first use by an end user. In some embodiments the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 6 days. In some embodiments the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after exposure to the cell culture medium for at least 6 days. In some embodiments the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after
15 autoclave treatment at 121°C for 40 minutes. In some embodiments the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 400 minutes.

[0017] In some embodiments the semiconductor substrate has a plurality of zones wherein at least a first zone is sensitive to an analyte, and a second zone that is insensitive to an analyte.

[0018] One aspect of the invention is an analyte-sensing system comprising: a working electrode having
20 a semiconductor surface that has immobilized thereon a redox-active moiety, wherein the redox-active moiety has an oxidation potential and/or reduction potential that is sensitive to the presence of said analyte; a counter electrode and optionally a reference electrode; a source for supplying a plurality of potentials to the working electrode; and a device for measuring current through the working electrode at the plurality of potentials.

25 [0019] In some embodiments the invention further comprises a second working electrode comprising a second semiconductor substrate comprising a second redox-active moiety having an oxidation potential and/or reduction potential that is insensitive to the presence of said analyte. In some embodiments the source for supplying a plurality of potentials is a potentiostat capable of applying square waves for square wave voltammetry.

30 [0020] In some embodiments the invention further comprises a computation system that communicates with the device for measuring current, and that calculates a reduction or oxidation potential from the measured current at a plurality of potentials.

[0021] In some embodiments the system is used as an in-line sensor in a process.

[0022] In some embodiments the currents measured at a plurality of potentials are used to determine
35 analyte concentration, and the determined analyte concentration is used to control a process parameter.

[0023] In some embodiments the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.1 pH unit. In some embodiments the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.01 pH units.

[0024] In some embodiments the sensor is capable of measuring analyte concentration without calibration with an external standard. In some embodiments the sensor remains sensitive to the analyte without calibration after a first use by an end user. In some embodiments the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 6 days.

In some embodiments the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after exposure to the cell culture medium for at least 6 days. In some embodiments the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 40 minutes. In some embodiments the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 400 minutes.

[0025] One aspect of the invention is a semiconductor substrate having a surface, wherein the surface comprises a redox-active moiety immobilized thereon, said redox-active moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of an analyte.

[0026] In some embodiments immobilized thereon is also a second redox-active moiety having an oxidation potential and/or a reduction potential that is substantially insensitive to the presence of the analyte.

[0027] In some embodiments the analyte is an ion. In some embodiments the analyte is hydrogen ion.

[0028] In some embodiments the semiconductor comprises an inorganic semiconductor. In some embodiments the semiconductor comprises an organic semiconductor. In some embodiments the inorganic semiconductor comprises silicon or gallium arsenide. In some embodiments the organic semiconductor comprises polyacetylene, polythiophene, or polypyrrole.

[0029] In some embodiments the semiconductor comprises silicon. In some embodiments the semiconductor comprises unpolished silicon. In some embodiments the semiconductor comprises polished silicon.

[0030] In some embodiments the redox-active moiety is directly bound to the surface. In some embodiments the redox-active moiety is covalently bound to the surface. In some embodiments the redox-active moiety is covalently bound to the surface through a linker. In some embodiments the redox-active moiety is covalently bound to a polymer that is immobilized onto the surface of the semiconductor substrate. In some embodiments the redox-active moiety is covalently bound to a polymer that is covalently bound to the surface of the semiconductor substrate

[0031] In some embodiments the semiconductor is doped. In some embodiments the semiconductor is N-doped. In some embodiments the semiconductor is P-doped.

[0032] In some embodiments substrate comprises crystalline silicon wherein the surface displaying predominantly one crystalline plane.

[0033] In some embodiments the substrate has a plurality of zones wherein each zone comprises a redox-active moiety immobilized thereon, said redox-active moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of an analyte. In some embodiments a first zone comprises a redox moiety sensitive to a first analyte, and a second zone comprises a redox moiety sensitive to a second analyte. In some embodiments the invention further comprises a third redox-active

moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of a second analyte. In some embodiments the second analyte is ammonia, oxygen or carbon dioxide.

[0034] One aspect of the invention is a method for forming an analyte-sensitive semiconductor electrode, said electrode having a surface, the method comprising: immobilizing a redox-active moiety that is sensitive to the presence of an analyte onto the surface. In some embodiments immobilizing the redox-active moiety covalently binds the redox-active moiety to the surface. In some embodiments the redox-active moiety is covalently bound to the surface through a linker. In some embodiments immobilizing the redox-active moiety comprises hydrosilation, a free radical reaction, carbodiimide coupling, a Diels-Alder reaction, a Michael addition, or click chemistry.

[0035] In some embodiments the redox-active moiety is covalently bound to a polymer that is immobilized onto the surface.

[0036] In some embodiments the step of immobilizing comprises polymerization including a monomer or oligomer comprising a redox-active moiety. In some embodiments the polymerization of a monomer or oligomer comprising a redox-active moiety includes a reaction with a functional group covalently bound to the surface, whereby the polymer formed by polymerization is covalently bound to the surface. In some embodiments the monomer or oligomer is electropolymerized onto the surface.

[0037] In some embodiments the step of immobilizing comprises coating or casting the polymer onto the surface.

[0038] In some embodiments the semiconductor surface comprises a composite electrode that comprises semiconductor particles within a matrix.

[0039] One aspect of the invention is a method of forming a semiconductor surface derivatized with one or more redox-active moieties comprising: contacting an H-terminated semiconductor surface with the one or more redox-active moieties wherein at least one redox active moiety is sensitive to the presence of an analyte, and wherein each redox-active moiety comprises a functional group that will react with the H-terminated semiconductor surface to form a covalently bond, thereby forming a derivatized semiconductor surface.

[0040] In some embodiments the semiconductor surface comprises silicon.

[0041] In some embodiments at least two redox active moieties are used, and one of the redox active moieties is insensitive to the presence of the analyte.

[0042] In some embodiments the H-terminated semiconductor surface is formed by treatment with hydrofluoric acid.

[0043] In some embodiments the functional group is a vinyl group. In some embodiments the functional group is an aldehyde group. In some embodiments the functional group is a diazonium group.

[0044] One aspect of the invention is a method of determining the concentration of an analyte, comprising placing an electrode in contact with said analyte, said electrode comprising a semiconductor substrate with a semiconductor surface having immobilized thereon an analyte-sensitive redox-active moiety, said analyte-sensitive redox-active moiety exhibiting an oxidation potential and/or reduction potential that is sensitive to the concentration of the analyte; applying a plurality of potentials to the

electrode; and measuring the current through the electrode at the plurality of potentials to determine a reduction and/or oxidation potential of the analyte-sensitive redox-active moiety, thereby determining the concentration of the analyte.

[0045] In some embodiments the measuring of the current at the plurality of potentials provides a peak current, and whereby the peak current is used to determine reduction and/or oxidation potential of the analyte-sensitive redox-active moiety.

[0046] In some embodiments the analyte is hydrogen ion.

[0047] In some embodiments the invention further comprises an analyte-insensitive redox-active moiety bound to an electrode comprising a semiconductor substrate, such redox-active moiety having a reduction and/or oxidation potential that is substantially insensitive to the analyte, further comprising determining the oxidation and/or reduction potential of the analyte-insensitive redox-active moiety, and determining the concentration of the analyte from the difference in the oxidation and/or reduction potentials of the analyte-sensitive and analyte-insensitive moieties.

[0048] In some embodiments the analyte is provided in a solution.

[0049] One aspect of the invention is a method comprising: measuring a pH value of a step in a water or waste treatment process with a voltammetric pH sensor, wherein the pH sensor comprises a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion; and using the pH value to monitor or control the treatment process.

[0050] One aspect of the invention is a method comprising: measuring a pH value of a reaction mixture in an biopharmaceutical process with a voltammetric pH sensor, wherein the pH sensor comprises a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration to obtain a pH value; and using the pH value to monitor the biopharmaceutical process.

[0051] In some embodiments the pH value is measured on a sample obtained from the reaction mixture.

[0052] One aspect of the invention is a reactor for carrying out a biopharmaceutical process wherein the reactor comprises a pH sensor having a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration.

[0053] In some embodiments the pH sensor is a voltammetric pH sensor.

[0054] In some embodiments the reactor is a disposable bioreactor. In some embodiments the reactor is a bioprocess flexible container.

[0055] One aspect of the invention is a method for carrying out an industrial process comprising: measuring a pH value of a step of an industrial process with a voltammetric pH sensor having a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration; and using the pH value to carry out the industrial process.

[0056] One aspect of the invention is a sensor for measuring ion concentration in a bodily fluid within a body comprising: an electrode configured to be in contact with a bodily fluid, said electrode comprising a

semiconductor surface that has immobilized thereon a redox-active moiety, wherein the redox-active moiety has an oxidation potential and/or reduction potential that is sensitive to concentration of said ion.

[0057] One aspect of the invention is a method of measuring concentration in a bodily fluid within a body comprising: placing such a sensor in contact with the bodily fluid; and operating the sensor to yield a value of the concentration of the ion present in said bodily fluid.

[0058] One aspect of the invention is a bioreactor comprising a reservoir for containing a reaction mixture and a pH probe wherein the pH probe comprises an electrode having a semiconductor surface, the semiconductor surface having immobilized thereon a redox active moiety having a reduction and/or oxidation potential that is sensitive to the presence of hydrogen ion.

[0059] In some embodiments the invention further comprises a semiconductor surface having immobilized thereon a redox active moiety having a reduction and/or oxidation potential that is insensitive to the presence of hydrogen ion.

[0060] In some embodiments the semiconductor surface on which the redox active moiety having a reduction and/or oxidation potential that is sensitive to the presence of hydrogen ion is immobilized is the same semiconductor surface on which the redox active moiety having a reduction and/or oxidation potential that is insensitive to the presence of hydrogen ion is immobilized on. In some embodiments the probe further comprises a counter electrode.

BRIEF DESCRIPTION OF THE DRAWINGS

[0061] Figure 1(a) shows a blow up drawing illustrating an embodiment of the invention comprising a semiconductor electrode sensor in a housing assembly. Figure 1(b) shows an exemplary housing assembly comprising the semiconductor electrode sensor.

[0062] Figure 2 depicts an embodiment of the invention comprising a unit that electrically connects to the semiconductor electrode sensor and comprising a source for supplying a plurality of potentials and a current measuring device.

[0063] Figure 3 depicts an embodiment of the invention comprising a probe for measuring analytes within a reactor comprising two working silicon electrodes.

[0064] Figure 4 illustrates a method of preparing of H-terminated silicon surface (Si-H).

[0065] Figure 5 illustrates a silicon surface derivatization with ferrocene moieties, vinyl-ferrocene (VFc) and ferrocene carboxaldehyde (FcA) by covalent attachment.

[0066] Figure 6 illustrates a silicon surface derivatized with anthracene moieties, vinyl anthracene (VA) and anthraldehyde (AnA) by covalent attachment.

[0067] Figure 7 illustrates a silicon surface derivatized with both the anthracene (VA) and ferrocene (VFc) moieties by covalent attachment.

[0068] Figure 8 depicts a schematic diagram and picture of an exemplary electrochemical cell.

[0069] Figure 9 depicts square wave voltammograms showing the effect of pH on VFc derivatized silicon sample at pH solution of 1.23, 4.61, 7.33 and 9.33.

[0070] Figure 10(a) depicts square wave voltammograms showing the effect of pH on VA derivatized silicon sample at pH solutions of 1.23, 4.61, 7.33 and 9.33. Figure 10(b) depicts a plot of peak potential against pH using the VA derivatized silicon sample.

[0071] Figure 11 depicts: (a) square wave voltammograms showing the effect of pH on VA + VFc derivatized silicon sample at pH solutions of 1.23, 4.65, 5.52 and 9.52; and (b) a plot of peak potential difference against pH using the VA+VFc derivatized silicon sample.

[0072] Figure 12 depicts square wave voltammograms showing the effect of 10 autoclave cycles on FcA + VA derivatized silicon sample. The electrochemical measurements were conducted at pH 6.52 buffer prior to autoclave and after autoclave.

[0073] Figure 13 depicts square wave voltammograms showing minimal fouling on FcA + AnA derivatized silicon samples. The electrochemical measurements were conducted at pH 6.52 buffer before and after six days exposure in the cell culture at four different FcA + AnA derivatized silicon samples (a), (b), (c) and (d).

[0074] Figure 14 depicts a square wave voltammogram obtained at FcA + AnA derivatized silicon sample in cell culture medium after sterilization and 6 days exposure.

[0075] Figure 15(a) depicts square wave voltammetric responses FcA on Si(100, N-type, 1-5 mΩ cm) in pH 7.33 buffer medium, showing every 50th scan of the 2,500 consecutive runs.; Figure 15(b) depicts voltammetric responses of VFc on Si(111, N-type, 0.02-0.05 Ω cm in pH 7.33 buffer medium, showing every 50th scan of the 2,500 consecutive runs.

[0076] Figure 16 depicts the square wave voltammetric response of Ac derivatized silicon surface at various temperatures (8, 17, 28 44, 56°C) in pH 7.33 buffer medium.

[0077] Figure 17 is a drawing of an embodiment of a bioreactor of the invention comprising a silicon-based voltammetric sensor.

[0078] Figure 18(a) depicts voltammograms taken on an anthracene derivatized silicon sensor over the 7 day period in cell culture medium (every 250th scan of the 10,000 consecutive runs). Figure 18(b) depicts a plot of the anthracene peak potential over the 7 day time period.

[0079] Figure 19 has charts showing the peak current of silicon substrates derivatized with (a) vinyl ferrocene and (b) ferrocene carboxaldehyde in pH 1.63 solution for four types of doped silicon.

[0080] Figure 20 depicts square wave voltammograms obtained with a four electrode system having highly-doped N-type silicon derivatized with vinyl ferrocene and at a lightly-doped P-type silicon derivatized with anthracene carboxaldehyde.

[0081] Figure 21 depicts an exemplary embodiment of a probe of the present invention having a four electrode configuration with a ring reference electrode (RE) and a ring counter electrode (CE). Figure 21(a) is a shaded drawing, and Figure 21(b) is a line drawing.

DESCRIPTION OF THE INVENTION

[0082] The invention relates to compositions, devices, systems, and methods for producing and using semiconductor electrodes modified with redox-active agents as sensors. The subject devices and systems

are particularly useful for voltammetrically measuring concentrations of an analyte of interest. The sensors of the present invention utilize a semiconductor electrode comprising redox-active species on its surface. At least one redox-active species on the semiconductor surface has a redox potential (reduction potential or oxidation potential) that is sensitive to the presence and or amount of an analyte of interest.

5 Voltammetry can be performed on the semiconductor electrode and used to measure the redox potential of the analyte-sensitive redox groups on the surface of the electrode. The measured value of the redox potential can then be used to determine the concentration of an analyte, for example an analyte in solution. In some embodiments, the semiconductor electrode of the present invention has more than one redox-active species. In one aspect and at least one redox-active species is sensitive to an analyte and
10 another redox-active species is insensitive to the presence of the analyte. Another aspect of the invention relates to the measurement of the concentration of hydrogen ion, or pH using the subject devices or systems. The surface modified semiconductor sensors of the present invention can be used to measure the pH of a variety of solutions. The surface modified sensors of the present invention are robust, reliable, accurate, and/or can be made such that they do not require calibration.

15 **[0083]** Semiconductors have advantages as substrates and sensors for the methods of the present invention. Semiconductor surfaces, for example, inorganic semiconductors such as silicon and organic semiconductors, can be amenable to surface modification, e.g. covalent modification. Semiconductors generally have electronic band structures, the characteristics of which can be modified, for example, by doping. In some cases, the semiconductor that is used is silicon. An advantage of using silicon as a
20 substrate and as an electrode is that silicon is amenable to mass production. In particular, semiconductor processing techniques are readily available for producing silicon electrodes in large quantities at low cost. In addition, existing semiconductor processing techniques make it feasible to integrate electronic functionality into the material comprising the silicon electrode. Another advantage of silicon is that it can form strong covalent bonds, for example with carbon, nitrogen, oxygen, thus allowing for the facile
25 and robust modification of the surface in a manner required for its intended uses. For instance, a silicon surface can be modified to attachment of any suitable redox-active agent. Silicon is also an advantageous surface for carrying out voltammetry in that it is stable to a wide range of electrical potential without undergoing degradation.

[0084] One aspect of the present invention is a surface modified semiconductor redox sensor that can
30 measure analyte concentration reliably and consistently with minimal intervention. One aspect of the invention is a semiconductor redox sensor that does not require calibration or re-calibration. The ability to sense analytes, such as hydrogen ion without calibration has a number of advantages for analyte measurements, for example, for in-line monitoring. For example, it allows for ease of operator handling for single point measurements. In some embodiments, the sensors of the present invention are included in
35 in-line operator-independent control measurements. Such in-line measurements can be made independent of the operator and can be used for process control, for example for pH measurements for process control. The subject sensors can be set to provide real-time measurements of an analyte, including, but not limited to real time-measurements of hydrogen ion concentration.

Semiconductor Substrate

[0085] The semiconductor substrate can comprise any suitable semiconductor material including those known in the art and those described herein. The semiconductor substrate can be an inorganic semiconductor or an organic semiconductor. The semiconductor substrate can be doped or undoped. In some embodiments the semiconductor substrate comprises silicon.

[0086] A semiconductor substrate of the invention is generally a solid material that has electrical conductivity in between that of a conductor and that of an insulator. The conductivity can vary over that wide range either permanently or dynamically.

[0087] Inorganic semiconductor substrates of the invention can comprise, for example, group IV elemental semiconductors, such as diamond (C), silicon (Si), germanium (Ge); group IV compound semiconductors, such as silicon carbide (SiC), silicon germanide (SiGe), group III-V semiconductors, such as aluminum antimonide (AlSb), aluminum arsenide (AlAs), aluminum nitride (AlN), aluminum phosphide (AlP), boron nitride (BN), boron phosphide (BP), boron arsenide (BAs), gallium antimonide (GaSb), gallium arsenide (GaAs), gallium nitride (GaN), gallium phosphide (GaP), indium antimonide (InSb), indium arsenide (InAs), indium nitride (InN), indium phosphide (InP); group III-V ternary semiconductor alloys, such as aluminum gallium arsenide (AlGaAs, $\text{Al}_x\text{Ga}_{1-x}\text{As}$), indium gallium arsenide (InGaAs, $\text{In}_x\text{Ga}_{1-x}\text{As}$), indium gallium phosphide (InGaP), aluminum indium arsenide (AlInAs), aluminum indium antimonide (AlInSb), gallium arsenide nitride (GaAsN), gallium arsenide phosphide (GaAsP), aluminum gallium nitride (AlGaN), aluminum gallium phosphide (AlGaP), indium gallium nitride (InGaN), indium arsenide antimonide (InAsSb), indium gallium antimonide (InGaSb), group III-V quaternary semiconductor alloys, such as aluminum gallium indium phosphide (AlGaInP, also InAlGaP , InGaAlP , AlInGaP), aluminum gallium arsenide phosphide (AlGaAsP), indium gallium arsenide phosphide (InGaAsP), Aluminum indium arsenide phosphide (AlInAsP), aluminum gallium arsenide nitride (AlGaAsN), indium gallium arsenide nitride (InGaAsN), indium aluminum arsenide nitride (InAlAsN), gallium arsenide antimonide nitride (GaAsSbN), group III-V quinary semiconductor alloys, such as gallium indium nitride arsenide antimonide (GaInNAsSb), gallium indium arsenide antimonide phosphide (GaInAsSbP), II-VI semiconductors, cadmium selenide (CdSe), cadmium sulfide (CdS), cadmium telluride (CdTe), zinc oxide (ZnO), zinc selenide (ZnSe), zinc sulfide (ZnS), zinc telluride (ZnTe), group II-VI ternary alloy semiconductors, such as cadmium zinc telluride (CdZnTe , CZT), mercury cadmium telluride (HgCdTe), mercury zinc telluride (HgZnTe), mercury zinc selenide (HgZnSe), group I-VII semiconductors, such as cuprous chloride (CuCl), group IV-VI semiconductors, such as lead selenide (PbSe), lead sulfide (PbS), lead telluride (PbTe), tin sulfide (SnS), tin telluride (SnTe), group IV-VI ternary semiconductors, such as lead tin telluride (PbSnTe), Thallium tin telluride (Tl_2SnTe_5), thallium germanium telluride (Tl_2GeTe_5), group V-VI semiconductors, such as bismuth telluride (Bi_2Te_3), and group II-V semiconductors, such as cadmium phosphide (Cd_3P_2), cadmium arsenide (Cd_3As_2), cadmium antimonide (Cd_3Sb_2), zinc phosphide (Zn_3P_2), zinc arsenide (Zn_3As_2), zinc antimonide (Zn_3Sb_2),

[0088] The inorganic semiconductor substrates of the invention can also comprise layered

semiconductors, such as lead(II) iodide (PbI₂), molybdenum disulfide (MoS₂), gallium selenide (GaSe), tin sulfide (SnS), bismuth sulfide (Bi₂S₃), other semiconductors, such as copper indium gallium selenide (CIGS), platinum silicide (PtSi), bismuth(III) iodide (BiI₃), mercury(II) iodide (HgI₂), thallium(I) bromide (TlBr), and miscellaneous oxides, such as titanium dioxide: anatase (TiO₂), copper(I) oxide (Cu₂O), copper(II) oxide (CuO), uranium dioxide (UO₂), uranium trioxide (UO₃).

[0089] In some embodiments of the invention, the semiconductor substrate can comprise an organic semiconductor. The organic semiconductor is any suitable organic material that has semiconductor properties. The organic semiconductor substrates of the invention can comprise, for example, small molecules, short chain (oligomers) and long chain (polymers). Examples of semiconducting small molecules (e.g. unsaturated and aromatic hydrocarbons) are pentacene, anthracene, rubrene, tetracene, chrysene, pyrene, perylene, coronene; metal complexes of porphine and phthalocyanine, compounds such as zinc 1,10,15,20-tetraphenyl-21 H, 23 H-porphine, copper phthalocyanine, lutetium bisphthalocyanine, and aluminum phthalocyanine chloride can be used. Suitable derivatives of these small molecules can also be used. In some embodiments, the organic semiconductor substrates of the invention can comprise thin films.

[0090] Examples of semiconducting polymers or oligomers include suitable conjugated hydrocarbon or heterocyclic polymers or oligomers. Suitable polymers or oligomers include: polyaniline, polypyrrole, and polythiophene, poly(3-hexylthiophene), poly(p-phenylene vinylene), F8BT, polyacetylene, polydiacetylene, polyacene, polyphenylene, poly(phenylene vinylene), polyfuran, polypyridine, poly(thienylene vinylene), poly(ferrocenyl vinylene phenylene vinylene), poly(fluorine), and poly(carbazole), and combinations thereof. Derivatives of these polymers, for instance derivatives having functional side chains amenable to the attachment of redox active species, can be used.

[0091] Other examples of semiconducting polymers are poly(anilinesulfonic acid), poly(ferrocenyl vinylene phenylene vinylene), poly(fluorine), and poly(carbazole). The organic semiconductor substrates of the invention can comprise, for example, organic charge-transfer complexes, and "linear backbone" polymers derived from polyacetylene, such as polyacetylene itself, polypyrrole, and polyaniline. Charge-transfer complexes can exhibit similar conduction mechanisms to inorganic semiconductors. This includes the presence of a hole and electron conduction layer and a band gap. The materials can exhibit tunneling, localized states, mobility gaps, and phonon-assisted hopping. Organic semiconductors can be doped. In some embodiments, the invention can utilize highly doped organic semiconductors, for example Polyaniline (Ormecon) and PEDOT:PSS. In some cases organic semiconductors can be produced such that they are transparent and/or flexible, which can be useful in some embodiments.

[0092] The semiconductors of the invention can typically be characterized as having a band gap, the band gap representing the amount of energy separating the valence and conduction bands of the semiconductor. The addition of dopants makes the band gap smaller, tending to allow more facile promotion of electrons from the valence band to the conduction band. A smaller band gap can result in higher conductivity for the semiconductor substrate. The band gap and conductivity characteristics of the

semiconductor substrates can be controlled in some cases by the introduction of dopants. In some cases upon the addition of a sufficiently large proportion of dopants, the semiconductor substrates of the invention can conduct electricity nearly as well as metals. Depending on the kind of dopant or impurity, a doped region of semiconductor can have more electrons or holes, and is named N-type or P-type semiconductor material, respectively. Junctions between regions of N- and P-type semiconductors create electric fields, which cause electrons and holes to be available to move away from them, and this effect is critical to semiconductor device operation. Also, a density difference in the amount of impurities produces a small electric field in the region which is used to accelerate non-equilibrium electrons or holes.

[0093] In some embodiments the presence of the band gap can be advantageous. For example, when performing electrochemistry, when the Fermi energy of a doped semiconductor lies at the same energy as the solution/molecular redox potential at a certain potential, generally no net transfer of charge/current will flow from the redox species (immobilized on the surface or in the solution) to the substrate or from the substrate to the redox species. This potential is sometimes referred to as the flatband potential. The location of the flatband potential can be influenced by dopant densities. The Mott-Schottky equation (see P. Schmuki, H. Bohni and J. A. Bardwell, J. Electrochem. Soc., 1995, 142, 1705) can be used to estimate the flat band potential.

[0094] A conductor electrode generally does not have a flatband potential thus has a broad potential window where current can flow from the redox species to the substrate. For example, when the conductor electrode is exposed to an aqueous solution containing a mixture of several redox active species, currents corresponding to these redox species (i.e. non-specific adsorption) will be recorded unless additional efforts are made to screen off these non-specific interactions, e.g. putting a diluent layer onto the conductor electrode. The non-specific interactions can be reduced or eliminated by using a semiconductor electrode wherein the semiconductor has a the band gap that has a limited potential window, such that the semiconductor can only conduct current for the electrochemical reaction occurring within that limited window. For example, the limited window can be between -1.0 to 0 V. Thus, in some embodiments, non-specific interactions in the solution can be reduced or eliminated by using a semiconductor electrode of the invention with the appropriate dopant density (which can be estimated using the Mott-Schottky equation).

[0095] One useful aspect of the semiconductors of the invention is that their conductivity can be modified by introducing impurities (dopants) into their crystalline lattice or amorphous regions. The process of adding controlled impurities to a semiconductor can be referred to as doping. The amount of impurity, or dopant, added to an intrinsic (pure) semiconductor alters its level of conductivity. Doped semiconductors are often referred to as extrinsic. The semiconductors of the present invention can be either intrinsic or extrinsic semiconductors.

[0096] Suitable dopants can be chosen, as is known in the art, on the atomic properties of the dopant and the material to be doped. In general, dopants that produce the desired controlled changes are classified as either electron acceptors or donors. A donor atom that activates (e.g., becomes incorporated into the

crystal lattice) donates weakly-bound valence electrons to the material, creating excess negative charge carriers. These weakly-bound electrons can move about in the semiconductor relatively freely and thus can facilitate electrical conduction in the presence of an electric field. In some cases, the donor atoms introduce some states below, but very close to, the conduction band edge. Electrons at these states can be thermally excited to conduction band, becoming free electrons, in some cases, at room temperature. In some embodiments an activated acceptor dopant is utilized. The activated acceptor can produce a hole. Semiconductors doped with donor impurities are typically called N-type, while those doped with acceptor impurities are typically known as P-type. In some embodiments of the invention, the semiconductor of the invention can have both donor and acceptor dopants. In some embodiments the semiconductor can have both n type and p type charge carriers. The n or p type designation generally indicates which charge carrier acts as the material's majority carrier. The opposite carrier is generally called the minority carrier, which, in some cases, exists due to thermal excitation at a lower concentration than the majority carrier.

[0097] As described herein, where the semiconductor is a group IV semiconductor such as silicon or germanium, suitable electron donors include, for example, phosphorous, arsenic, antimony, and bismuth.

In some embodiments the dopant is phosphorous. In some embodiments the dopant is antimony. Where the semiconductor is a group IV semiconductor such as silicon or germanium, suitable electron acceptors include boron, aluminum, gallium, and the like. In some embodiments, the dopant is boron. Where the semiconductor comprises an element in another group than group IV, as is known in the art, elements that are outside of that group can act as either n type or p type dopants.

[0098] In addition to modification through doping, the resistance of semiconductors can in some cases, for example, be modified dynamically by applying electric fields. The ability to control resistance/conductivity in semiconductor substrate or within regions of semiconductor substrate dynamically through the application of electric fields can be useful in some embodiments.

[0099] The semiconductor substrate can be in any form that is amenable to the production of a semiconductor electrode. The semiconductor substrate can comprise a monolithic piece of the semiconductor, a coating of the semiconductor deposited onto another material, or a powder of semiconductor particles. The semiconductor substrate can be a monolithic form such as a chip, wafer, rod, needle, block, ingot, or the like. The semiconductor substrate can alternately be in particulate form, for example in the form of powder comprised of particles. The particles can be of arbitrary shape or can be in the form of fibers, sheets, beads, discs, or balls. Where the substrate is in the form of a powder made up of particles, it will generally be formed into a composite electrode as described in more detail below.

[00100] The semiconductor substrate of the present invention can be a thin layer of semiconductor that is formed upon another material, for example a thin layer of semiconductor formed on glass would constitute a semiconductor substrate.

[00101] In some embodiments the semiconductor substrate used to make the electrode is a composite material comprising semiconductor particles dispersed in a matrix or binder. The semiconductor substrate can be made of a composite material comprising a powder of semiconductor dispersed in a

binder to make a composite semiconductor substrate. The semiconductor powder can be in the form of spheres, crystallites, rods, fibers, or any other arbitrary shape. In one embodiment the composite electrode is made of semiconductor crystallites dispersed in a polymeric matrix. The matrix or binder can be an organic, inorganic, or organometallic polymer. Non-limiting examples of useful inorganic polymeric materials include polyphosphazenes, polysilanes, polysiloxane, polygermanes, polymeric sulfur, polymeric selenium, silicones, and mixtures of any of the foregoing.

[00102] In some embodiments the polymer can be an organic polymer. Non-limiting examples of suitable organic polymeric materials include, but are not limited to, thermoset materials and thermoplastic materials. Non-limiting examples of polymers useful in the invention include polyesters such as polyethylene terephthalate, polybutylene terephthalate, and polyethylene naphthalate, polycarbonates, polyolefins such as polyethylene, polypropylene, and polyisobutene, acrylic polymers such as copolymers of styrene and an acrylic acid monomer, and polymers containing methacrylate, polyamides, thermoplastic polyurethanes, vinyl polymers, polyimides, polyamides, polytetrafluoroethylene and other fluoropolymers, and mixtures of any of the foregoing.

[00103] The binder can be insulating, semiconductive, or conductive. In one embodiment, the binder is a material, such as a polymer, which is an insulating material. Where an insulating binder is used, the current will tend to only flow through the dispersed semiconductor powder. In some embodiments, the binder includes conductive components. In some embodiments, the binder comprises a conductive polymer such as polyaniline, polyacetylene, poly(alkylthiophene), poly(alkylpyrrole), and the like. In some embodiments, the conductive component can comprise conductive particles such as metal particles, such as nickel particles other conductive particles including carbon particles. In some embodiments, the conductive component is chosen such that the conductive component such as the conductive polymer exhibits reduction and/or oxidation potentials that are outside of the reduction and/or oxidation potentials of the redox active moieties.

[00104] The composite semiconductor substrate can be formed by mixing the semiconductor powder with a monomer, oligomer, or prepolymer and curing the monomer, oligomer or prepolymer to form a polymeric matrix. The polymerization can be initiated in any manner known in the art or disclosed herein. The polymerization can be initiated, for example, thermally or photochemically in the presence of an initiator. The polymerization can be carried out with one or more crosslinkers. The cross-linkers can be chosen to adjust the physical properties of the polymeric matrix and thus adjust the properties of the composite semiconductor substrate. The composite semiconductor substrate can be formed by mixing the semiconductor powder with a molten thermoplastic polymer, forming the substrate, and allowing the mixture to cool. The composite semiconductor substrate can be formed by mixing the semiconductor powder with a polymer or prepolymer in a solvent, and allowing the solvent to evaporate to form the composite. Combinations of any of the above methods can be used.

[00105] The electrical properties of the composite semiconductor substrate can be affected by the amount of semiconductor, the particle size, and the particle shape. In general, the amount of semiconductor in the composite is high enough to create conductive pathways throughout the material. This amount of material

necessary to provide conductive paths across the material is sometimes called the percolation threshold.

The amount of semiconductor particles for conductivity can also depend on the processing conditions such as the viscosity of the binder and the amount of mixing. The amount of semiconductor is generally set at a level at which the physical properties of the material, such as mechanical strength and flexibility will not suffer to the point that the material is not useful. The amount of semiconductor will generally be from 0.1 volume percent to 70 volume percent of the composite material. In some embodiments the amount of semiconductor will be from 1 volume percent to 50 volume percent. In some embodiments the amount of semiconductor will be from 10 volume percent to 40 volume percent. The amount of semiconductor can be from 1% to 5%, 5% to 10%, 10% to 15%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50% or 50% to 60%.

[00106] The composite semiconductor substrate can be formed by methods used for shaping polymeric materials such as coating, molding, and casting into shapes that are useful as electrodes. The composite semiconductor substrate electrode will generally be connected to an electrically conductive wire to apply current and potential. The material can be cast, coated, and/or molded onto a conductive substrate such as a metal to form a conductive junction for connecting conductors for transfer of current to and from the composite electrode.

[00107] The semiconductor substrates of the invention generally have a high enough electrical conductivity to act as electrodes, to transmit current for the oxidation and/or reduction of the bound redox-active moieties. To make the semiconductor substrate more conductive, the semiconductor substrate can include impurities or dopants to increase electrical conductivity and reduce the resistivity.

[00108] The electrical resistivity (reciprocal of conductivity) can be for example 0.1 (ohm-centimeters), 1 (ohm-centimeters), 10 (ohm-centimeters), 100 (ohm-centimeters), to in excess of 1000 or even 10,000 (ohm-centimeters) or even higher which is comparable to graphite and conventional metallic conductors.

[00109] In some embodiments the resistivity of the semiconductor substrate is the range of 0.0001 to 100,000 Ω -cm (or ohm-centimeters). In some embodiments the resistivity of the semiconductor substrate is the range of 0.001 to 10,000 Ω -cm. In some embodiments the resistivity of the semiconductor substrate is the range of 0.01 to 1000 Ω -cm. In some embodiments the resistivity of the semiconductor substrate is the range of 0.1 to 100 Ω -cm. In some embodiments the resistivity of the semiconductor substrate is within the range of 1 to 100 Ω -cm. In some embodiments the resistivity of the semiconductor substrate is within the range of 10 to 90 Ω -cm. In some embodiments the semiconductor substrate is single crystal semiconductor is Si(100) that is P-type with a resistivity of 10 to 90 Ω -cm. In some cases several semiconductor substrates with different resistivities will be used. For example, a system of the invention may comprise one lightly doped semiconductor substrate having one redox active species bound to it, and also a more highly doped semiconductor surface having another redox active species bound to it. For example, a system of the invention may comprise one lightly doped semiconductor surface having a pH sensitive redox active moiety such as anthraquinone bound thereto, and a second semiconductor surface that is more highly doped having a hydrogen ion insensitive redox active moiety such as ferrocene bound to it.

[00110] In some cases different regions of the semiconductor substrate can be doped at different levels.

For example, it is well known in the semiconductor processing art that a mask can be used to cover some regions of the semiconductor, while leaving other regions exposed. The exposed regions can be treated selectively with dopants resulting in a semiconductor surface wherein some regions are doped differently than other regions. By using multiple steps with various masks, the conductivity properties of different regions of the semiconductor surface can be controlled. Thus, some regions can have high conductivity, some low, some regions can have a large band gap, and other regions can have small band gaps, some regions can have N-doping, some P-doping, and some no doping. In addition, the various regions can be connected with conducting regions, for example deposited metal, e.g. gold in to be able to electrically address the various regions.

Silicon Substrate

[00111] In some aspects of the invention, the semiconductor substrate comprises silicon. One aspect of the invention is a silicon substrate having a surface onto which are attached redox-active moieties. The silicon substrate can comprise amorphous silicon or silicon comprising a variety of crystalline forms. The silicon substrate can also be polycrystalline. In some embodiments the silicon substrate can have both amorphous and crystalline regions. In some embodiments, the silicon can be nanocrystalline or microcrystalline silicon. Nanocrystalline silicon and microcrystalline silicon can be used to describe an allotropic form of silicon with paracrystalline structure having small grains of crystalline silicon within the amorphous phase. Where the silicon substrate is crystalline, the surface of the silicon substrate can have various crystalline faces on the surface. Crystalline silicon is generally in a face centered cubic (fcc) form. In some embodiments, such as where a polycrystalline silicon is used, the surface may have multiple crystalline planes exposed. Where single crystal silicon is used, in some cases, the silicon substrate can be made to have one or more crystal planes represented or predominantly represented on the surface. In some embodiments, the surface of the silicon substrate will comprise one or more crystal planes having a crystalline lattice of (xxx) wherein x is an integer corresponding to the lattice defining the crystal plane. In some embodiments the crystal planes (100), (010), (001), (110), (101), or (112) will be predominantly represented at the surface. In some embodiments a silicon substrate has the (100) plane predominantly represented at the surface.

[00112] The silicon electrode of the present invention can comprise a polished or an unpolished silicon substrate. Silicon is generally polished prior to silicon processing, for example, building features such as transistors. In some embodiments, such as those embodiments where electronic functionality is incorporated into a silicon sensor, a polished silicon surface may be desirable. In other embodiments, an unpolished silicon substrate can be used. An unpolished silicon substrate can be less expensive than a silicon substrate that has gone through a polishing step. In addition, an unpolished silicon substrate can have a higher surface area for a given area of silicon than a polished silicon substrate.

[00113] The silicon electrode of the present invention can comprise porous silicon. An advantage of porous silicon is an increase of the effective surface area. An increased surface area can be advantageous

for providing a higher signal from the oxidation and reduction of the surface bound redox moieties due to a higher number of such moieties in contact with the sample. As is known in the art, if the surface is too porous, it can become less robust. Therefore the level of porosity can be controlled to maximize important properties for the particular applications. The porous silicon can be prepared by, for example, galvanostatic, chemical, or photochemical etches from silicon wafers. In some embodiments, chemical etching with hydrofluoric acid (HF) can be used to produce a porous silicon substrate. In some embodiments, the average pore size of the silicon substrate ranges from 1 nm to 500 nm. Pore size can be measured by, for example, nitrogen gas adsorption or Hg porosimetry. In some embodiments, the amount of porosity ranges between 1% and 98%. In some embodiments, the amount of porosity ranges between 5% and 75%. In some embodiments, the amount of porosity ranges between 10% and 50%. In some embodiments, the amount of porosity ranges between 20% and 40%. In some embodiments the porosity is between 1% to 5%, 5% to 10%, 10% to 30%, 20% to 40%, 30% to 50%, or 40% to 60%. The porosity measurement can be made on an area percent basis or a volume percent basis.

[00114] In addition, porous silicon could be readily integrated with existing silicon-based integrated circuit (IC) manufacturing processes.

[00115] The silicon substrate can be in any form that is amenable to the production of a silicon electrode. The silicon substrate can comprise a monolithic piece of silicon, a coating of silicon deposited onto another material, or a powder of silicon particles. The silicon substrate can be a monolithic form such as a chip, wafer, rod, needle, block, ingot, or the like. The silicon substrate can alternately be in particulate form, for example in the form of powder comprised of particles. The particles can be of arbitrary shape or can be in the form of fibers, sheets, beads, discs, polyhedra, or balls. Where the substrate is in the form of a powder made up of particles, it will generally be formed into a composite electrode as described in more detail below.

[00116] In some embodiments the semiconductor electrode is made from single crystal silicon. The single crystal silicon can be made by zone melting, also called zone refining, a process in which rods of metallurgical grade silicon are first heated to melt at one end. Then, the heater is typically slowly moved down the length of the rod, keeping a small length of the rod molten as the silicon cools and re-solidifies behind it. Since most impurities tend to remain in the molten region rather than re-solidify, when the process is complete, most of the impurities in the rod will typically have been moved into the end that was the last to be melted. This end is then cut off and discarded, and the process repeated if a still higher purity is desired. The single crystal silicon of the invention can also be produced via the Czochralski process, (CZ-Si) which tends to be inexpensive and is capable of producing large size crystals.

[00117] In some embodiments the silicon electrode is polycrystalline. As used herein, the term "polysilicon" is used interchangeably with the term "polycrystalline silicon". In some embodiments, the polysilicon is deposited. The polycrystalline silicon can be deposited by low pressure chemical vapor deposition (LPCVD), plasma-enhanced chemical vapor deposition (PECVD), or solid-phase crystallization (SPC) of amorphous silicon in certain processing regimes. These processes can require relatively high temperatures, for example, above 300°C. The polycrystalline silicon electrodes can also

be made, for example on polymeric substrates, using laser crystallization to crystallize a precursor amorphous silicon (a-Si) material on a plastic substrate without melting or damaging the plastic. In some cases, the for example, short, high-intensity ultraviolet laser pulses are used to heat the deposited a-Si material to above the melting point of silicon, without melting the entire substrate. By controlling the temperature gradients, the crystal size on the electrodes can be controlled. Grain sizes can be, for instance from 10 nanometer to 1 micrometer. Another method to produce polysilicon at low temperatures for the electrodes of the present invention is a metal-induced crystallization in which an amorphous silicon thin film is crystallized, for example at temperatures at or above 150°C, while in contact of a metal film such as aluminum, gold, or silver. The polycrystalline silicon electrodes can also be formed onto a metal structure such as a wire. For example, the end of a cylindrical wire can be coated with polysilicon, which can be derivatized with redox active species as described herein. The structure can be used as an electrode or portion of an electrode with silicon portion accessible to the medium containing the analyte, and the wire acting to connect the silicon electrode to the parts of the system providing voltage and allowing for the flow of current.

[00118] An advantage of polysilicon over amorphous silicon (a-Si) is that the mobility of the charge carriers can be orders of magnitude larger than in single crystal silicon and the material also can show greater stability under electric field and light-induced stress.

[00119] The silicon substrate of the present invention can be a thin layer of silicon that is formed upon another material, for example a thin layer of silicon formed on glass would constitute a silicon substrate.

[00120] In some embodiments the silicon substrate used to make the electrode is a composite material comprising silicon particles dispersed in a matrix or binder. The silicon substrate can be made of a composite material comprising a powder of silicon dispersed in a binder to make a composite silicon substrate. The silicon powder can be in the form of spheres, crystallites, rods, fibers, or any other arbitrary shape. In one embodiment the composite electrode is made of silicon crystallites dispersed in a polymeric matrix. The matrix or binder can be an organic, inorganic, or organometallic polymer. Non-limiting examples of useful inorganic polymeric materials include polyphosphazenes, polysilanes, polysiloxane, polygermanes, polymeric sulfur, polymeric selenium, silicones, and mixtures of any of the foregoing.

[00121] In some embodiments the polymer can be an organic polymer. Non-limiting examples of suitable organic polymeric materials include, but are not limited to, thermoset materials and thermoplastic materials. Non-limiting examples of polymers useful in the invention include polyesters such as polyethylene terephthalate, polybutylene terephthalate, and polyethylene naphthalate, polycarbonates, polyolefins such as polyethylene, polypropylene, and polyisobutene, acrylic polymers such as copolymers of styrene and an acrylic acid monomer, and polymers containing methacrylate, polyamides, thermoplastic polyurethanes, vinyl polymers, polyimides, polyamides, polytetrafluoroethylene and other fluoropolymers, and mixtures of any of the foregoing.

[00122] The binder can be insulating, semiconductive, or conductive. In one embodiment, the binder is a material, such as a polymer, that is an insulating material. Where an insulating binder is used, the current

will tend to only flow through the dispersed silicon powder. In some embodiments, the binder includes conductive components. In some embodiments, the binder comprises a conductive polymer such as polyaniline, polyacetylene, poly(alkylthiophene), poly(alkylpyrrole), and the like. In some embodiments, the conductive component can comprise conductive particles such as metal particles, such as nickel particles other conductive particles including carbon particles. In some embodiments, the conductive component is chosen such that the conductive component such as the conductive polymer exhibits reduction and/or oxidation potentials that are outside of the reduction and/or oxidation potentials of the redox active moieties.

[00123] The composite silicon substrate can be formed by mixing the silicon powder with a monomer, oligomer, or prepolymer and curing the monomer, oligomer or prepolymer to form a polymeric matrix. The polymerization can be initiated in any manner known in the art or disclosed herein. The polymerization can be initiated, for example, thermally or photochemically in the presence of an initiator. The polymerization can be carried out with one or more crosslinkers. The cross-linkers can be chosen to adjust the physical properties of the polymeric matrix and thus adjust the properties of the composite silicon substrate. The composite silicon substrate can be formed by mixing the silicon powder with a molten thermoplastic polymer, forming the substrate, and allowing the mixture to cool. The composite silicon substrate can be formed by mixing the silicon powder with a polymer or prepolymer in a solvent, and allowing the solvent to evaporate to form the composite. Combinations of any of the above methods can be used.

[00124] The electrical properties of the composite silicon substrate can be affected by the amount of silicon, the particle size, and the particle shape. In general, the amount of silicon in the composite is high enough to create conductive pathways throughout the material. This amount of material necessary to provide conductive paths across the material is sometimes called the percolation threshold. The amount of silicon particles required for conduction can also depend on the processing conditions such as the viscosity of the binder and the amount of mixing. The amount of silicon is generally set at level at which the physical properties of the material, such as mechanical strength and flexibility will not suffer to the point that the material is not useful. The amount of silicon will generally be from 0.1 volume percent to 70 volume percent of the composite material. In some embodiments the amount of silicon will be from 1 volume percent to 50 volume percent. In some embodiments the amount of silicon will be from 10 volume percent to 40 volume percent. The amount of silicon can be from 1% to 5%, 5% to 10%, 10% to 15%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50% or 50% to 60%.

[00125] The composite silicon substrate can be formed by methods used for shaping polymeric materials such as coating, molding, and casting into shapes that are useful as electrodes. The composite silicon substrate electrode will generally be connected to an electrically conductive wire to apply current and potential. The material can be cast, coated, and/or molded onto a conductive substrate such as a metal to form a conductive junction for connecting conductors for transfer of current to and from the composite electrode.

[00126] The silicon substrates of the invention generally have a high enough electrical conductivity to act as electrodes, and to transmit current for the oxidation and/or reduction of the bound redox-active moieties. To make the silicon substrate more conductive, the silicon substrate will generally include impurities or dopants to increase electrical conductivity. Where polycrystalline silicon is used, the

polycrystalline silicon electrode can either be deposited as doped polycrystalline silicon (in situ doped) or can be deposited undoped and subsequently doped with an impurity dopant such as phosphorus or boron by ion implantation or a thermal diffusion process. Dopant impurities, such as phosphorus and boron, tend to diffuse much more rapidly along the grain boundaries than they do through the silicon itself.

[00127] The dopant for a type IV semiconductor such as silicon, can be, for example, either an electron donor or an electron acceptor. Suitable electron donors are phosphorous, arsenic, antimony, and bismuth. In some embodiments the dopant is phosphorous. In some embodiments the dopant is antimony. Suitable electron acceptors are boron, aluminum, gallium, and the like. In some embodiments, the dopant is boron. In some embodiments, electron acceptors can impart a chemical resistance to the silicon electrode.

[00128] Where the silicon substrate is a monolithic material such as a wafer, the dopant can be either distributed throughout the bulk of the silicon, or can be limited to the surface region of the silicon wafer. In some embodiments, for example where the silicon substrate comprises multiple zones with different redox active moieties, the dopant can be distributed such that isolated regions of the surface of the silicon substrate are conductive.

[00129] The dopant is generally present in an amount greater than 0.01 weight percent of the silicon, and generally in an excess of 0.1 percent of the silicon. Generally, the dopant is less than 3 weight percent of the silicon, and almost always less than 6 weight percent of the silicon. The presence of small amounts of the dopant can increase the electrical conductivity. The dopant may not be homogeneously distributed throughout the silicon, and the local concentration may vary between different regions of the silicon material.

[00130] The electrical resistivity (reciprocal of conductivity) can be for example 0.1 (ohm-centimeters), 1 (ohm-centimeters), 10 (ohm-centimeters), 100 (ohm-centimeters), to in excess of 1000 or even 10,000 (ohm-centimeters) or even higher which is comparable to graphite and conventional metallic conductors.

[00131] In some embodiments the resistivity of the silicon substrate is the range of 0.0001 to 100,000 Ω -cm. In some embodiments the resistivity of the silicon substrate is the range of 0.001 to 10,000 Ω -cm. In some embodiments the resistivity of the silicon substrate is the range of 0.01 to 1000 Ω -cm. In some embodiments the resistivity of the silicon substrate is the range of 0.1 to 100 Ω -cm. In some embodiments the resistivity of the silicon substrate is within the range of 1 to 100 Ω -cm. In some embodiments the resistivity of the silicon substrate is within the range of 10 to 90 Ω -cm. In some embodiments the silicon substrate is single crystal silicon is Si(100) that is P-type with a resistivity of 10 to 90 Ω -cm. In some cases several silicon substrates with different resistivities will be used. For example, a system of the invention may comprise a one lightly doped silicon substrate having one redox active species bound to it, and also a more highly doped silicon surface having another redox active

species bound to it. For example, a system of the invention may comprise one lightly doped silicon surface having a pH sensitive redox active moiety such as anthraquinone bound thereto, and a second silicon surface that is more highly doped having a hydrogen ion insensitive redox active moiety such as ferrocene bound to it.

5 [00132] In some cases different regions of the silicon substrate can be doped at different levels. For example, it is well known in the semiconductor processing art that a mask can be used to cover some regions of the silicon, while leaving other regions exposed. The exposed regions can be treated selectively with dopants resulting in a silicon surface wherein some regions are doped differently than other regions. By using multiple steps with various masks, the conductivity properties of different regions of the silicon surface can be controlled. Thus, some regions can have high conductivity, some low, some regions can have a large band gap, and other regions can have small band gaps, some regions can have N-doping, some P-doping, and some no doping. In addition, the various regions can be connected with conducting regions, for example deposited metal, e.g. gold to be able to electrically address the various regions.

15 [00133] In some embodiments, Nisil is used. Nisil is an alloy of nickel and silicon. In some embodiments Nisil with 4% - 5% silicon is used. In some embodiments Nisil with 4.4% silicon is used. In some embodiments Nicrosil is used. Nicrosil is a nickel alloy. In some cases Nicrosil comprising 14.4% chromium, 1.4% silicon, and 0.1% magnesium is used.

[00134] In some cases, for example, where the silicon substrate is cast silicon, the silicon substrate will include, for example, a silicide of a transition metal to provide castability. The silicide of the transition metal can provide favorable mechanical properties to the cast alloy. Typical metals useful in providing the transition metal silicide present in the silicon electrode of the secondary cell of this invention include titanium, zirconium, hafnium, vanadium, columbium, chromium, molybdenum, tungsten, manganese, iron, cobalt, nickel, copper, ruthenium, rhodium, palladium, osmium, iridium, platinum, gold, and silver.

25 Most commonly, the transition metal present as the silicide in the silicon alloy will be a silicide of manganese, chromium, iron, cobalt, nickel, or molybdenum. The amount of the silicide will be sufficient to provide satisfactory castability but not great enough to deleteriously effect the properties of the silicon, i.e., from 2 percent or more up to as high as 30 percent transition metal, elemental basis.

30 *Redox-active moiety*

[00135] In one embodiment, the subject semiconductor surface comprises a surface modified with redox-active functional groups. At least one redox-active functional group on the surface is sensitive to the presence and or the level of a substance in the solution. In some embodiments, the semiconductor surface will have at least one redox-active functional group sensitive to an analyte, and at least one redox-active functional group that is substantially insensitive to the analyte to be tested. When used in this manner, the substantially insensitive group can act as a reference, allowing for greater accuracy and reproducibility of the measurements.

[00136] The redox groups can be chemically or physically bound to the surface. The redox groups can be attached to the semiconductor covalently, can be adsorbed to the semiconductor, or can be attached to polymers that are either covalently or non-covalently bound to the surface. Covalent binding of either the redox group or the polymer to which the redox group is a part can be beneficial in improving the lifetime and stability of the electrode. Semiconductor materials such as silicon and germanium can form covalent bonds with carbon, and thus is a desirable substrate for functionalizing with carbon based molecules. The covalent binding to the surface can be through a bond between the semiconductor, e.g. silicon, and carbon, oxygen, nitrogen, sulfur, or other atom. In some embodiments the bond is between silicon and carbon. In some embodiments the bond is between silicon and oxygen. The physical bonding can occur through adsorption, and can include, for example, spontaneous self assembly onto the semiconductor surface of molecules such as those derived from fatty acids which comprise redox active moieties.

[00137] Where a linker group is used, the linker can be small, for example one to 3 atoms, or can be longer, e.g. 20 to 100 atoms, or can be any size between large and a small linker. Where a short linker is used, the redox-active moiety is held close to the surface. Where a longer group is used, the redox active moiety may be able to move away from the surface, for example into the solution. Linker groups can comprise hydrophilic, hydrophobic groups, or mixtures thereof. Linker groups can comprise, for example, hydrocarbons, esters, ethers, amides, amines, carbonyls, thiols, olefins, silicones, or other organic, inorganic or organometallic groups. The linker groups can be formed by polymerization or oligomerization reactions such as free radical, cationic, or anionic polymerization. The linker group can comprise, for example, ethylene oxide, propylene oxide, vinyl ether, or acrylamide repeat units. Linkers can have ring structures including aromatic rings. The variation in the linker structure can be used to vary the mobility of the redox-active moiety in the solution.

[00138] As used herein, the term moiety generally refers to a portion of a molecule or substituent. A redox-active moiety may be highly substituted, and can still act as a redox-active moiety. As used herein, the terms "redox active moiety", "redox active group", "redox active functional group", and "redox group" are used interchangeably. Thus, for example, the redox-active moiety ferrocene includes substituted ferrocenes, ferrocene polymers, and ferrocene covalently attached to the surface with or without linker molecules.

[00139] In some embodiments, the redox-active moiety can be incorporated into a polymer, and the polymer comprising the redox active moiety can be immobilized onto the semiconductor surface. The immobilization of the polymer can be either chemical or physical. The immobilization of the polymer can be through covalent bonds, or through adsorption of the polymer to the semiconductor surface.

[00140] In some embodiments, the redox-active moiety is bound to a particle that is bound to semiconductor. The particle is generally an electrically conductive particle. The particle attached to the semiconductor surface in a manner that allow for current to flow between the semiconductor surface and the particle. The particles can be attached chemically or physically to the surface. For example, the redox-active moiety can be attached to a carbon particle, and the carbon particle attached to the semiconductor surface. In some embodiments, the carbon particle can be a carbon nanotube. In some

embodiments of the invention, carbon nanotubes can be attached to the surface of the semiconductor, where there are redox-active groups attached to the carbon nanotubes. For instance, attachment of well-aligned single-walled carbon nanotubes architecture to a single-crystal silicon surface can be used. In some embodiments, for example, ferrocenemethanol molecules are attached to single walled carbon nanotube (SWCNT) arrays that are directly anchored to the silicon surface, for example, a (100) surface. For example, single wall carbon nanotubes can be coupled to the surface using this method as described in Yu et al., *Electrochimica Acta* 52 (2007) 6206-6211.

[00141] The redox-active moieties generally have reversible redox activity with well-defined cyclic voltammetry oxidation and/or reduction peaks. A suitable reference redox reagent can vary from application to application or medium to medium depending on the intended use.

[00142] The position of the reduction and/or oxidation potentials of the redox active moiety can be chosen to improve the accuracy and quality of the measurement of redox potential. In some cases, the reduction and or oxidation potential can be chosen to be away from other redox active species. The silicon surface, for example, generally has a wide window in which to perform measurement of reduction or oxidation potential without interfering with the measurement of the reduction and or oxidation of the redox active moieties bound to the surface. The silicon surface can generally be used to measure oxidation and/or reduction potentials from between negative 2 V to positive 2 V. In some cases, for example where the medium is an aqueous medium, the reduction and/or oxidation potential of the redox-active moiety can be chosen so as not to fall within the reduction or oxidation potential of the medium to minimize interference. This can be useful where cyclic voltammetry is used, and is less important when square wave voltammetry is used.

[00143] Redox-active moieties that are insensitive to the presence of analytes should show little or no change in their oxidation and/or reduction potentials in the presence or absence of such analytes. Redox-active moieties that are generally insensitive to the presence of analytes, and in particular are insensitive to the presence of hydrogen ion include: ferrocene, polyvinylferrocene, Os(bpy)₂Cl₂, Ru(bpy)₂Cl₂, viologen, polyviologen, and polythiophene. Redox reagents having a high degree of electrochemical reversibility are generally preferred. Figure 5 shows examples of the hydrogen ion insensitive redox-active moiety ferrocene bound to a silicon surface.

[00144] Non-limiting redox-active moieties that are sensitive to hydrogen ion include: quinones, anthroquinones, phenanthroquinones, phenylene diamines, catechols, phenothiazinium dyes, and monoquaternized N-alkyl-4,4'-bipyridinium. In some embodiments the redox-active moiety that is sensitive to the presence of hydrogen ion can include inorganic materials and metal oxides. Hydrogen ion sensitive inorganic redox-active inorganic moieties include Prussian Blue, Ni(OH)₂, and RuO_x. Figure 6 shows examples of the hydrogen ion sensitive redox-active moiety anthracene covalently bound to a silicon surface. Figure 7 shows an example of a silicon surface having covalently bound thereto both the hydrogen ion sensitive redox-active moiety ferrocene and the hydrogen ion insensitive redox-active agent anthracene.

[00145] In some embodiments the analyte is carbon monoxide (CO). An example of a CO sensitive redox-active agent is ferrocenyl ferrazetene disulfide or ferrazetene-ferrazetene disulfide. A CO insensitive redox-active agent can be, for example, ferrocene.

[00146] In some embodiments the analyte is an alkali metal. Alkali metal sensitive redox-active agents include, for example: 1,1'-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7,16-diyl dimethyl), ferrocenyl thiol, and other ferrocene derivatives containing covalently attached cryptands. These materials are described, for example, Hammond, et al., J. Chem. Soc. Perkin. Trans. I 707 (1983); Medina, et al., J. Chem. Soc. Chem. Commun. 290 (1991); Shu and Wrighton, J. Phys. Chem. 92, 5221 (1988). Included are examples such as the above ferrocenyl ferrazetene and ferrocenyl cryptand, in which an ordinarily chemically insensitive redox center (ferrocene) is covalently linked to a chemical recognition site in such a way as to make the redox center chemically sensitive. Also suitable are molecules or polymers in which the sensor and reference functionalities are covalently linked such as 1-hydro-1'-(6-(pyrrol-1-yl)hexyl)-4,4'-bipyridinium bis(hexafluorophosphate), as described by Shu and Wrighton, J. Phys. Chem. 92, 5221 (1988).

[00147] In some cases, more than one analyte-sensitive redox-active moiety can be used. For example, there can be one redox-active moiety that is sensitive to hydrogen ion, and another that is sensitive to a second analyte such as CO, oxygen, ammonia, or an alkali metal. This approach allows for the simultaneous measurement of several analytes. In some cases redox-active agents sensitive to 3 or more analytes can be used. Where there are redox agents sensitive to multiple analytes, in many cases, it is also desired to have one or more analyte-insensitive redox-active moieties bound to the semiconductor surface as well to provide a reference, to improve accuracy, and to minimize or avoid calibration.

[00148] Where more than one redox-active moiety is used it can be important to ensure there is not significant interference between the peaks. This can be desirable especially when the multiple redox-active moieties are on the same electrically addressable zone on the semiconductor substrate. This can be achieved, for example, by ensuring that there is sufficient separation of the oxidation and reduction potentials, or by physically separating the redox moieties.

[00149] In some embodiments, the semiconductor substrate has a plurality of isolated separately electrically addressable zones. In some embodiments, the different zones will comprise different redox-active moieties. The use of separate zones can be beneficial in that the voltammetric measurements can be carried out separately, allowing for the use of multiple redox-active agents with that have similar reduction and/or oxidation potentials.

[00150] The separately electrically addressable zones can be made by conventional semiconductor processing methods, for example masking to create structures in specific areas on the surface, for example specific areas on the surface having certain levels of doping. Conventional semiconductor processing can also be used to incorporate conductive interconnects allowing the zones to be separately addressable. Masking can also be used during the attachment of the redox-active moieties to the semiconductor surface to attach specific redox active moieties to different regions of the surface.

[00151] The semiconductor substrate with separately addressable zones can effectively create an

electrochemical sensor array. One aspect of the invention is a semiconductor electrochemical sensor array wherein a plurality of zones, each zone comprising a redox active moiety. The array can have multiple zones with analyte-sensitive redox-active moieties, and one or more zones with analyte-insensitive redox active moieties. A zone can have a single redox-active moiety, or multiple redox active moieties. The array can be constructed, for example, to measure both pH and O₂, wherein one zone comprises a redox-active moiety sensitive to hydrogen ion, another zone has a redox active moiety sensitive to O₂, and a third zone with a redox-active moiety that is insensitive to both hydrogen ion and O₂.

[00152] In some embodiments, an array of separately addressable zones or an array of electrodes is used where there are a plurality of zones or electrodes that are each constructed to measure the same analyte and used in a redundant matter, wherein another zone capable of measuring the same analyte is used either simultaneously or in place of the other zone or electrode. In some cases, more than one zone or electrode, for example in an array, is used simultaneously to improve the quality of the measurement. In some cases, more than one zone or electrode, for example in an array, is used sequentially, wherein if one zone or electrode shows degraded performance, the measurement of that analyte is performed on another zone or electrode constructed to have similar characteristics. The sequential use of similar zones or electrodes can provide reliability of measurement over time. While the electrodes of the present invention can be prepared to be robust and to resist fouling, in some circumstances, degradation of the measurement over time may occur. In some embodiments, the maximum current ($I_{\max}$) can be monitored over time. A system can be configured, for example, such that when the maximum current drops below a certain level, a switch is made to a redundant zone or electrode for further measurement of that analyte. There can be multiple redundant elements, e.g. 1, 2, 3, 4, 5, 10, 20 or more redundant elements.

[00153] The semiconductor substrate of the invention can also comprise circuitry. The circuitry can be used, for example for controlling the current and potential provide to the redox-active moiety. The circuitry can also be used for analyzing signals or for processing data related to the voltammetric measurement. The circuitry can also have other functionality, such as the ability to measure other parameters such as temperature, the ability to store data, or the ability to send data and receive instructions from a remote location. The circuitry can contain, for example, an amplifier such as an operational amplifier. The circuitry can contain, for example, an analog to digital converter (ADC). In some cases having the amplifying and ADC functions incorporated into the semiconductor substrate can provide higher quality and reliability of the transmission of the signal from the sensor. The use of circuitry on the semiconductor substrate can be particularly useful when an array of zones is utilized. The circuitry can assist in managing the passage of current in and out of the semiconductor substrate. In some cases, the circuits can allow for schemes to simultaneously or sequentially address the zones, for example, by mutiplexing (MUX).

[00154] An aspect of the invention is the incorporation of the semiconductor substrate described above into a semiconductor electrode sensor. The semiconductor electrode sensor comprising the semiconductor substrate can be used for the measurement of the presence or absence of one or more analytes, or can be used to accurately measure the concentration of analyte in a sample.

[00155] The semiconductor electrode sensor of the invention can be used to measure analytes including hydrogen ion, alkali metals, CO, or O₂. In some embodiments, the semiconductor electrode sensor is used to measure the concentration of hydrogen ion, or pH.

[00156] The semiconductor electrode sensor comprises a semiconductor substrate as described above comprising a redox-active moiety that is sensitive to the presence of an analyte. The semiconductor electrode sensor may also comprise a redox active moiety that is insensitive to the presence of an analyte. The semiconductor electrode sensor can comprise more than one semiconductor substrate. For example, the sensor may comprise one semiconductor substrate that has an analyte sensitive redox active moiety and another semiconductor substrate having an analyte insensitive redox active moiety.

[00157] The semiconductor electrode sensor is configured to be incorporated into a system that will supply voltage, and can drive current to the sensor to perform voltammetry. Thus, the semiconductor substrate or substrates within the sensor will be electrically connected in a manner which will allow for connection to a device for supplying and measuring current and voltage.

[00158] The semiconductor electrode sensor is generally the working electrode in an electrochemical system that will also comprise a counter electrode, and in some embodiments, a reference electrode.

[00159] The sensor will be put into contact with a sample having the analyte to be detected. The sample is generally a liquid sample. In some cases the sample can be a gel, suspension, molten, or semi-solid medium. The sample can be, for example, a foodstuff. The sample can be any type of liquid including hydrocarbons, oils, fluorocarbons, silicones, and aqueous solutions. Where the analyte is hydrogen ion, an aqueous medium is generally used, but in some case a polar protic medium or polar aprotic medium can be used. The sensor is useful for measuring pH in aqueous solutions.

[00160] In some embodiments, the sensor of the invention can accurately measure analyte concentrations where the analyte is present in a concentration range from 10⁻¹ M to 10⁻¹⁴ M. In some embodiments, the sensor of the invention can accurately measure analyte concentrations where the analyte is present in a concentration range from 10⁻³ M to 10⁻¹⁰ M. In some embodiments, the sensor of the invention can measure the concentration to an accuracy of plus or minus 100%, 50%, 30%, 20%, 10%, 5%, 2% or 1%. In some embodiments, the sensor of the invention can measure the concentration within a range of 10⁻³ M to 10⁻¹⁰ M to an accuracy of plus or minus 100%, 50%, 30%, 20%, 10%, 5%, 2% or 1%.

[00161] In some embodiments, the analyte is hydrogen ion, and the sensor of the invention can accurately measure the pH in a range from pH1 to pH14. In some embodiments, the analyte is hydrogen ion, and the sensor of the invention can accurately measure the pH in a range from pH3 to pH10. In some embodiments, the sensor of the invention can accurately measure pH to an accuracy of plus or minus 0.5, 0.3, 0.2, 0.1, 0.07, 0.05, 0.03, 0.02, or 0.01 pH units. In some embodiments, the sensor of the invention

can accurately measure pH in a range from pH3 to pH10 to an accuracy of plus or minus 0.5, 0.3, 0.2, 0.1, 0.07, 0.05, 0.03, 0.02, or 0.01 pH units.

[00162] The semiconductor electrode sensors of the invention can accurately measure analyte concentration in a wide variety of sample types. The sensors can be made to be robust, and resistant to fouling, and therefore reliable for long-term measurements.

[00163] One aspect of the invention is a sensor which does not require routine calibration, or in some cases, any calibration at all. Conventional potentiometric sensors rely on a glass membrane to sense, for example, hydrogen ion. These types of sensors generally need to be calibrated on a regular basis, usually by placing the sensor into standards of known analyte concentration. These types of sensors generally need calibration when going from one solution to another solution, and will also need calibration with time, even if kept within the same solution and upon standing outside of a solution. The situation is made worse if there is a change in the composition of the medium over the time that the sensor is monitoring the medium, for example, when monitoring a chemical reaction, biochemical reaction, or fermentation. In these cases, potentiometric sensors may drift and need calibration due to the accumulation of some species in the reaction, or due to fouling of the sensor by species present.

[00164] In some embodiments, the sensors of the present invention do not need to be calibrated under any of these situations. In some embodiments, the sensors of the invention do not need to be calibrated over time in solution. In some embodiments, the sensors of the invention do not need to be calibrated after an hour, 10 hours, 1 day, 2 days, 5 days, a week, two weeks, a month, 6 months, 1 year, 2 years or longer while in a solution or in storage. In some embodiments the sensors of the present invention are accurate at measuring analyte concentration to 50%, 40%, 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2% or 0.1% after the times above. In some embodiments where the sensors measure pH, the sensors are accurate to 1, 0.8, 0.5, 0.3, 0.2, 0.1, 0.08, 0.05, 0.03, 0.02, or 0.01 pH units after the times above. In some embodiments where the sensors measure pH, the sensors are accurate to within 0.1 pH units after one week in solution or in storage.

[00165] In some embodiments, the sensor is capable of measuring analyte concentration without any calibration with an external standard. In some embodiments, the sensor remains sensitive to the analyte without calibration after a first use by an end user.

[00166] In some embodiments the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 1, 3, 6, 9, 12, 18, or 24 hours or 2, 3, 4, 6, 8, 12, 24, 48, 60, 90, or more days. In some embodiments the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 3 days. In some embodiments the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 6 days. In some embodiments, the sensor is capable of measuring pH with an accuracy of 0.2 units after exposure to the cell culture medium.

[00167] In some embodiments, the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of 0.2 units after autoclave treatment at 121°C for 10, 20, 40, 80, 100, 200, 400, or 800 minutes. In some embodiments, the analyte is hydrogen ion and the sensor is capable of measuring pH

with an accuracy of 0.2 units after autoclave treatment at 121°C for 40 minutes. In some embodiments, the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of 0.2 units after autoclave treatment at 121°C for 400 minutes.

[00168] A subject sensor that does not require calibration over long periods of time in a medium that can change characteristics is useful, for example, as an implantable sensor. The implantable sensor can be placed under the skin or within the body in contact with a bodily fluid such as blood, saliva, breast milk, amniotic fluid, lymph, sweat, tears, or urine. The sensor can measure the concentration of analytes such as hydrogen ion, sodium, potassium, calcium, or oxygen.

[00169] The implantable sensor has an electrode configured to be in contact with a bodily fluid, said electrode comprising a semiconductor surface that has immobilized thereon a redox active moiety, wherein the redox active moiety has an oxidation potential and/or reduction potential that is sensitive to concentration of said ion. The implantable sensor can be included in an implantable medical device such as described in U.S. Patent 6,738,670. For example, the implantable medical device in which the sensor resides could include pacemakers, defibrillators, drug delivery pumps, diagnostic recorders, cochlear implants, and the like. The implantable medical device is typically programmed with a therapy and then implanted in the body typically in a subcutaneous pocket at a site selected after considering clinician and patient preferences. In some embodiments the implanted device is in a form which can be swallowed, allowing the measurement of the properties of the regions encountered as it passes through the body such as the digestive tract including the stomach, the upper and lower intestines, and the colon. The information obtained by the sensor in the implanted device can either be accessed in real time, for example, by wireless communication, or can be retrieved from the device after passage through the body. A wide variety of programmers, also known as downlink transmitters, can be used to transmit data to and receive data from the implantable medical device. Examples of downlink transmitters include devices such as physician programmers, patient programmers, programming wands, telemetry access units, and the like. The clinician, for example, can periodically use a physician programmer to communicate with the implantable medical device to manage the patient's therapy and collect implantable medical device data. The semiconductor electrode sensor can be incorporated into or attached to the implantable medical device and can provide data on analyte concentration within the region of the body into which it is implanted. The patient can use the patient programmer to communicate with the implanted device to make therapy adjustments that have been programmed by the clinician. Both the physician programmer and patient programmer can have an antenna locator that indicates when a telemetry head is aligned closely enough with the implanted device for adequate telemetry.

[00170] An aspect of the invention is a method of measuring concentration in a bodily fluid within a body comprising placing a semiconductor electrode sensor comprising a redox active moiety in contact with the bodily fluid; and operating the sensor to yield a value of the concentration of the analyte present in said bodily fluid.

System

[00171] One aspect of the invention is a system for measuring analyte concentration. In one embodiment, the system comprises: a working electrode having a semiconductor surface that has immobilized thereon a redox active moiety, wherein the redox active moiety has an oxidation potential and/or reduction potential that is sensitive to the presence of an analyte; a counter electrode and optionally a reference electrode; a source for supplying a plurality of potentials to the working electrode; and a device for measuring current through the working electrode at the plurality of potentials. The working electrode referred to herein can comprise the semiconductor electrochemical sensor described above. It is desirable in many embodiments that the semiconductor surface also has immobilized thereon a second redox active moiety having an oxidation potential and/or reduction potential that is insensitive to the presence of said analyte. The redox active moiety that is insensitive to the presence of the analyte can be on the same semiconductor surface, or can be on another surface in electrical contact with the system and in contact with the sample. The system is configured such that the working electrode, the counter electrode, and optionally the reference electrode are in contact with the sample. In many embodiments, the sample is a liquid sample, and the electrodes are each in contact with the liquid. In some cases, the sample will not be a liquid, but will be a solid, generally comprising a solid electrolyte, or a gas.

[00172] In some embodiments, the system will have two or more working electrodes. For example, in some embodiments, the system will have one working electrode comprising a semiconductor surface that has immobilized thereon a redox active moiety whose oxidation potential and/or reduction potential is sensitive to the presence of said analyte, and a second working electrode comprising redox active moiety whose oxidation potential and/or reduction potential is insensitive to the presence of said analyte. An example of a system with two working electrodes is a system having two semiconductor wafers or chips, one of which has a redox active moiety which is sensitive to pH, such as anthracene, and another redox active moiety which is insensitive to pH, such as a ferrocene. In some cases the semiconductor wafer or chip on which each redox active species is immobilized will be a different type of semiconductor wafer or chip. For instance, the semiconductor wafer or chip to which the pH sensitive moiety is bound may have one doping level, and the semiconductor wafer or chip on which the pH insensitive moiety is bound may have a different doping level. This type of construction can be beneficial because, in some cases, one type of redox active species will perform better in terms of amplitude, sensitivity or stability with one type of doping, while another redox active species will perform better on a semiconductor wafer or chip with a different type of doping. In some embodiments, the pH sensitive moiety, e.g. anthracene, is bound to a semiconductor wafer that has a low level of doping, and the pH insensitive moiety, e.g. ferrocene is bound to a silicon wafer that has a higher level of doping. In some embodiments the semiconductor wafer onto which the pH sensitive moiety, e.g. anthracene is bound has a resistivity between 1 Ω -cm to 1000 Ω -cm, or between 10 Ω -cm to 90 Ω -cm, or between 10 Ω -cm to 40 Ω -cm while semiconductor wafer onto which the pH insensitive moiety, e.g. ferrocene, is bound has a resistivity between 0.001 Ω -cm and 0.1 Ω -cm, or from 0.001-0.005 Ω cm resistivity, 0.02-0.05 Ω -cm. In some embodiments, an N-type semiconductor, e.g. silicon wafer is used for the pH insensitive moiety, e.g. ferrocene. In some

embodiments, an N-type semiconductor, e.g. silicon wafer is used for both the pH sensitive and the pH insensitive moiety.

[00173] In some embodiments, the system will have 3 or more working electrodes. For example, in some embodiments, the system will have one working electrode comprising a semiconductor surface that has immobilized thereon a redox active moiety that is sensitive to the presence of a first analyte, a second working electrode comprising a semiconductor surface that has immobilized thereon a redox active moiety that is sensitive to the presence of a second analyte, and a third working electrode comprising a semiconductor surface that has immobilized thereon a redox active moiety that is insensitive to the presence of either the first analyte nor the second analyte. The system can also have more than 3 working electrodes, for example having 4, 5, 6, 7, 8, 9, 10, 12, 20, 50 or more working electrodes, each having redox active moieties sensitive to and analyte. These systems can also have one or more than one semiconductor working electrode having a redox species that is insensitive to the analytes, for example to provide a reference. In some cases more than one redox species that is insensitive to the analyte can be used.

[00174] In some embodiments the system of the present invention comprises a probe that comprises the 2 or more electrodes. The probe can physically hold the electrodes such that the electrodes can be brought into contact with the sample. The probe allows the working electrodes to be held close to the reference and/or counter electrode. Figure 21 shows an exemplary embodiment of a probe of a system of the present invention having 4 electrodes: a first working electrode (WE1), a second working electrode (WE2), a reference electrode (RE), and a counter electrode (CE). Figure 21(a) shows a shaded drawing of the probe. Figure 21(b) shows a line drawing of the probe. As shown in Figure 21, in some cases it can be beneficial in the present invention for the reference electrode and the counter electrode to have a ring configuration. In other embodiments, only one of the reference electrode or counter electrode will have a ring configuration. A ring electrode can in some cases provide signal stability. While this embodiment shows one configuration, there are other ring electrode configurations that can be used with the present invention.

[00175] In some embodiments it is useful to use lightly doped semiconductor e.g. silicon wafers for a moiety such as anthracene. While not being bound by theory, the band gap of the semiconductor, e.g. silicon can be influenced by the level of doping of the semiconductor, and it is believed that in some cases, the use of a semiconductor with the appropriate level of doping can be useful to tailor the appropriate redox active moiety with the appropriate band gap. Thus in some cases it is desirable to use lightly doped semiconductor, e.g. silicon with a moiety such as anthracene. Thus in some embodiments it is useful to use two semiconductor working electrodes: one optimized for ferrocene moieties and the other optimized for the anthracene moieties. When two working electrodes are used, in some embodiments, two sequential electrochemical measurements (e.g. with square wave voltammetry) will be carried out using the same counter and reference electrode. For instance, the first measurement can be conducted using reference, counter and working electrode 1 (e.g. anthracene derivatized) between -1.2 to -0.5 V, followed by the second measurement which will be conducted using reference, counter and

working electrode 2 (e.g. ferrocene derivatized) between 0 to 0.5V. The peaks potential detected in the first and second measurements can then be stored and processed to get a pH reading. This type of system and method can be accomplished through the use of a bipotentiostat or a two-channel multiplexer. A similar approach can be applied to multiple working electrodes with a mutipotentiostat or a multi-channel multiplexer.

[00176] The system is configured to carry out voltammetric measurements on the sample. In one aspect the present invention provides a method which includes the measurement of pH with a voltammetric pH sensing system comprising the semiconductor electrode sensor described above, a potentiostat for providing voltage to the electrodes, and a meter for detecting the current as a function of voltage.

[00177] The counter electrode typically is needed to complete the electrochemical circuit to make the measurements described herein. The counter electrode is generally made of a material which is chemically inert to the medium so that its potential does not change significantly during the course of measurement. Suitable materials in many applications include platinum, gold, stainless steel, and carbon.

[00178] A reference electrode is optional and is used as a third electrode in some embodiments of the invention. In the case of a three-electrode system, the counter electrode generally completes the circuit, allowing current to flow through the cell, while the reference electrode maintains a constant interfacial potential difference regardless of the current. In the case where the system comprises an analyte sensitive redox active moiety and an analyte insensitive redox active moiety, the analyte insensitive redox active moiety can act as a reference, allowing the potential difference to be used to determine analyte concentration. Even where the system comprises an analyte insensitive moiety, in some embodiments, a reference electrode will still be used. In some embodiments, pseudo-reference electrodes can also be utilized. Reference electrodes that can be employed include: Standard hydrogen electrode (SHE), also known as "normal hydrogen electrode" (NHE), saturated calomel electrode (SCE), copper-copper(II) sulfate electrode, and silver/silver chloride (Ag/AgCl) electrode. In some embodiments a silver electrode can act as a silver/silver chloride electrode where sufficient chloride is present at or near the silver electrode. In some cases, e.g. in non-aqueous medium, a metal electrode such as a platinum or a silver electrode can be used as the reference electrode.

[00179] To carry out voltammetry, the system generally has a source for supplying a plurality of potentials. The voltammetry can be, for example cyclic voltammetry, pulse voltammetry, normal pulse voltammetry, square wave voltammetry, differential pulse voltammetry, linear voltammetry, or square wave voltammetry. The source for supplying a plurality of potentials can be a potentiostat, for example, a potentiostat capable of applying square waves for square wave voltammetry.

[00180] Generally, the analyte concentration is determined by using voltammetry to identify the position of current peaks, which current peaks indicate the reduction or oxidation potential of a redox active moiety. In some embodiments, the position of the reduction and/or oxidation potential of the analyte sensitive redox active moiety is used to determine the concentration of the analyte. This method can be used, for example, where no analyte insensitive redox active moiety is employed.

[00181] Where an analyte insensitive redox active moiety is used, detection is generally accomplished by measuring the potential difference, delta E, associated with current peaks for oxidation (or reduction) of the immobilized redox active moieties, where the magnitude of delta E can be related to the concentration of analyte, e.g. hydrogen ion (H⁺) in solution. The analyte insensitive redox active moiety has an electrochemical response that is insensitive to variations in the medium and serves as the reference. Current peaks for oxidation or reduction of the reference and indicator are determined from a voltammogram using a counter electrode.

[00182] In some embodiments, the system further comprises a computation system that communicates with the device for measuring current. The computation system can have algorithms for calculating reduction or oxidation potential from the measured current at a plurality of potentials from the voltammetry measurements. The computing systems can be part of the sensing system, in some cases allowing the sensing system to be self-contained. The computing system can comprise memory for storing raw and/or processed data from the sensors. The computing system can be connected to a transmission device that will wirelessly, by wire, fiber or other means, transmit processed data to an external device. The computing system can provide signals and measurements which can be transmitted in some cases in real time, allowing the system to alert end users of conditions which may require attention. The transmitted signals and measurements can, for example, provide the information required for adjusting a manufacturing process such as a chemical or biochemical process.

[00183] In some embodiments the system is made up of a housing that holds the semiconductor electrode sensor that is electrically connected to a unit comprising the source for supplying a plurality of potentials and the current measuring device. In some embodiments the unit also comprises the computing system described above for at least partially analyzing the data. The unit can be self powered, e.g. with a battery, or can have a connection to an outside power source. The unit can have a display and input buttons to allow the user to control the measurement and to read the output from the sensor. The unit can have transmission capability for sending out data, and for receiving instructions or to be tested by an external device.

[00184] Figure 1 shows drawings of an embodiment of the connection of a semiconductor sensor electrode into a sensor housing for use in measuring analytes in a fluid such as the fluid in a biochemical reactor. Figure 1(a) shows a blow up drawing of an assembly that holds the semiconductor electrode sensor and provides electrical connections to the semiconductor electrode sensor for voltammetry measurements. In Figure 1(a) the semiconductor electrode sensor (I), is held in place by the end cap (II), in contact with the metallized ceramic disk (III). The ceramic disk can be metallized in specific areas on both the front and the back of the disk, with vias connecting the specific metallized areas. On one side of the ceramic disk, the semiconductor working electrodes as well as the counter and optionally reference electrodes can be present. For example, electrodes such as the semiconductor working electrodes can each being mounted to a specific metallized area. The disk is then mounted into the housing such that the side of the disk having the electrodes is exposed to the medium into which the probe is immersed, and the other side of the disk is away from the medium, allowing for electrical connection to the metallized areas

on to the disk such that voltage can be applied and current can flow to and from the electrodes. The sealing gasket (IV) provides sealing from the fluid in which the sensor is immersed while allowing electrical contact with the pin contacts (V) on the shaft of the housing (VI). Figure 1(b) shows the housing assembled for insertion into the fluid to be measured. Pipe fittings are used to seal the wires that provide electrical connection to the semiconductor electrode for voltammetric measurements.

[00185] Figure 2 shows a drawing of an embodiment of the unit comprising the source for supplying a plurality of potentials and the current measuring device. Figure 2(a) shows a top view and Figure 2(b) shows a back side view. The unit has an electrical input/output connector for connecting to the electrodes for carrying out voltammetry (4/20 mA I/O). The unit has a connection for AC power. The unit has a universal serial bus interface (USB I/F) and an RS-232 Serial port for transmitting data, for receiving instructions, and for testing and debugging by an external device. The unit also comprises a liquid crystal display (LCD) and has user interface buttons to allow the user to control the measurements and to read the output from the sensor.

[00186] Figure 3 shows another exemplary embodiment of an aspect of the system of the present invention. Figure 3 shows a probe that contains two semiconductor (silicon) working electrodes, a reference electrode, and a counter electrode. The electrodes can be electrically connected through the probe to the source for supplying a plurality of potentials to the working electrode, the counter electrode, and optionally the reference electrode, and a device for measuring current through the working electrode at the plurality of potentials. In this embodiment, the two working electrodes, the reference electrodes and the counter electrode are contained on the end of the probe on a disk which allows the electrodes to be in contact with the medium comprising the analyte(s) to be measured. The areas of the various electrodes can be varied to improve the performance of the system. While this embodiment shows two working electrodes, in some embodiments, there will be one working electrode, and in other embodiments, there are 3, 4, 5, 10, 20, or more working electrodes. In some embodiments one working electrode can comprise a semiconductor surface with a redox active moiety that is sensitive to pH, such as anthracene, and the other working electrode can comprise a redox active moiety that is insensitive to pH, such as ferrocene. In some embodiments, the semiconductor surface on which the pH sensitive moiety, such as anthracene, is bound comprises a silicon wafer that is lightly doped, and the silicon surface on which the pH insensitive moiety, such as ferrocene, is bound comprises a silicon wafer that is more heavily doped.

[00187] The semiconductor working electrodes can be bonded to conductive regions on the disk. Conductive vias through the disk allow electrical connection to electrode pins which are contained within the housing. The electrode pins are in turn, electrically connected, for example to wires, which can run through the probe housing, and then out of the housing to electrically connect the electrodes to the source for applying potentials to the electrode, and to the device for measuring the current that passes through the electrodes. The threads, o-ring, and hex body allow for the probe to be mounted into a reactor such as a bioreactor or fermentor. The threads allow for the probe to mate with a corresponding threaded hole through the wall of the reactor, the hex body allows for tightening the probe into the reactor, and the

gasket assists in establishing a seal. In some embodiments the unit is mounted into the reactor without the use of threads, mounting without threads can provide resistance to some failure modes that are present when threads are used.

[00188] In some embodiments, the system is configured to be used as an in-line sensor in a process. An in-line sensor can be a sensor that is used in an on-going process. In some embodiments the sensor is in a vessel, in other embodiments the sensor is in a conduit or pipe through which a process fluid flows. In some embodiments, the currents measured at a plurality of potentials by voltammetry are used to determine analyte concentration, and the determined analyte concentration is used to control a process parameter. The systems of the present invention are valuable in in-line sensing in that they can be made to be robust, to resist fouling, and are able to measure analyte concentration for long periods of time in media that changes its properties, as in a process such as a chemical reaction.

Method of making a semiconductor electrochemical sensor

[00189] An aspect of the invention is a method of making an electrochemical sensor. The method generally comprises having a semiconductor substrate with a surface and immobilizing a redox active moiety with a reduction and/or oxidation potential that is sensitive to an analyte onto the semiconductor surface.

[00190] An aspect of the invention is a method for forming an analyte-sensitive semiconductor electrode, said electrode having a semiconductor surface, the method comprising: immobilizing a redox-active moiety that is sensitive to the presence of an analyte onto the semiconductor surface.

[00191] Any suitable method such as those known in the art or disclosed herein can be used to construct a semiconductor surface as described above that is useful as part of a subject sensor. The redox groups can be immobilized onto the surface chemically or physically. The redox groups can be reacted with the semiconductor surface to attach them to the semiconductor covalently. Alternatively, the redox active groups can be adsorbed to the semiconductor. The redox active groups can also be immobilized by attaching the groups to a polymer that is either covalently or non-covalently bound to the surface. Covalent binding of either the redox group or the polymer to which the redox group is a part to the surface can be beneficial in improving the lifetime and stability of the electrode.

[00192] Methods are known for covalent attachment of functional groups to semiconductors such as silicon or germanium. Silicon can, for example, form covalent bonds with carbon, and thus is a desirable substrate for functionalizing with carbon based molecules. The covalent binding to the surface can be through a bond between the semiconductor, e.g. silicon, and carbon, oxygen, nitrogen, sulfur, or other atom. In some embodiments the bond to the surface is between the semiconductor, e.g. silicon, and carbon. In some embodiments the bond to the surface is between semiconductor, e.g. silicon, and oxygen.

[00193] In some embodiments, the immobilization of the redox active moiety by covalent binding to the semiconductor surface is accomplished by reaction with a semiconductor hydride, for example, a silicon hydride (Si-H) surface. A semiconductor-hydride, e.g. silicon-hydride surface can be obtained, for

example by treatment of the semiconductor, e.g. silicon surface, for example a surface that is in the native oxide state, with hydrofluoric acid (HF). For example, dilute (1–3%) aqueous HF treatment, or, a 40% aqueous NH_4F treatment can be used to create a Si-H terminated surface. Porous silicon, when etched through standard procedures involving HF, can also be used as a Si-H surface. Figure 4 shows a

5 schematic illustrating the conversion of a native oxide surface to a Si-H surface on a silicon wafer through the treatment of the wafer with a 2.5% aqueous solution of HF. A Si-H surface can also be formed by other processes, for example by decomposition of silanes as described in U. S. Patent 6,444,326. A Si-H surface can also be formed through reacting surface silanol moieties with reagents such as trihydroxyhydridosilane via sol-gel type methods (see e.g. 5,017,540, and 5,326,738). A Germanium
10 hydride (Ge-H) surface can undergo the same types of the reactions of Si-H to create covalently bonded redox active reagents. Suitable reactions to covalently bond to the Si or Ge surface are described, for example, in J.M. Buriak, Chem. Review 2002, 102 (5), 1271. The hydrides of other semiconductor substrates can also be prepared and used to covalently bond redox active moieties. Suitable semiconductor hydrides include for example, hydrides of silicon germanium (M.S. Carroll, et al., J.
15 Electrochem Soc. 2000, 147 (12), 4652), gallium arsenide (P.E. Gee, et al., J. Vacuum Sci. Tech. A: Vacuum Surf. Film 1992, 10 (4), 892), gallium nitride, diamond film (S. Yamashita, et al., US 5,786,604 (1998)), and indium phosphide (Y. Sun, et al., J. Appl. Phys. 2005, 97, 124902).

[00194] The semiconductor-hydride surface, e.g. Si-H surface, can be reacted with a variety of functional groups to create covalent bonds and thereby attach a redox active moiety to the semiconductor surface.

20 The Si-H surface, Ge-H surface or other Semiconductor-H surface can participate in hydrosilylation reactions, involving the addition of, for example, the Si-H across an unsaturated site to form a Si-C, Si-O, or Si-N bond to the surface. Functional groups which can be used in these reactions including hydrosilylation include alkenes, alkynes, imines, carbonyls and oximes. These reactions, including hydrosilylation can be carried out thermally, photochemically, with a metal catalyst or with a radical
25 initiator (see Buriak, Chem Commun, 1999, 1051-1060). The Si-H surface or other semiconductor-H surface can also be reacted with alkyl or aryl carbanions through, for example, Grignard, or lithium reagents. In some embodiments, a Si-H surface or other semiconductor-H surface can react with azido, diazo, and diazonium groups. Suitable diazonium reactions are described, for example, in Stewart et al., J. Am. Chem. Soc. 126, 2004, 370-378.

30 [00195] Figure 5 illustrates the reaction of a surface Si-H with an aldehyde functionality attached to a ferrocene redox active moiety to create a covalently bound ferrocene through a Si-O bond. Figure 5 also illustrates the reaction of a surface Si-H with a vinyl functional group attached to a ferrocene redox active moiety to create a covalently bound ferrocene through a Si-C bond. Figure 6 illustrates the reaction of a surface Si-H with an aldehyde functionality attached to an anthracene redox active moiety to create a
35 covalently bound anthracene through a Si-O bond. Figure 6 also illustrates the reaction of a surface Si-H with a vinyl functional group attached to an anthracene redox active moiety to create a covalently bound anthracene through a Si-C bond. Figure 7 illustrates the reaction of both a ferrocene redox active moiety and an anthracene redox active moiety through vinyl functionality to produce a silicon surface with a

covalently attached redox group that is sensitive to hydrogen ion (anthracene) and a covalently attached redox group that is insensitive to hydrogen ion (ferrocene). In some embodiments, a carbonyl group such as an aldehyde group is substituted for the vinyl functionality for providing attachment to the surface.

[00196] The redox active moieties can alternatively be attached covalently to the surface by direct reactions with a semiconductor, e.g. silicon surface from which all functionality has been removed, typically by high temperature and vacuum. The pure silicon surface, for example, can react directly, for example with alkenes and alkynes to form Si-C covalent attachment. (see Bateman, et. al., *Angew. Chem. Int. Ed.* 1998, 37(19), 2683-2685). Diazonium species can also be used to functionalize the surface either thermally or electrochemically. In some embodiments, ultrahigh vacuum techniques can be used to prepare the functionalized surfaces of the invention, for example by [2 + 2] Reactions of alkynes and alkenes or Diels–Alder ([4 + 2]) reactions of dienes with reconstructed Si surfaces.

[00197] Capping of semiconductor oxide surfaces such as silica and glass surfaces with alkyl-, alkoxy- and chloro-silanes may also be used to functionalize the semiconductor surface.

[00198] The semiconductor surface can contain oxide functionality including hydroxy functionality (native oxide). In some embodiments, the semiconductor electrodes of the invention are modified by covalent attachment to this oxide functionality. For example, the hydroxy groups of silicon or other semiconductor elements can be coupled to surface bound groups using the many reactions known in organic chemistry for carbon bound hydroxy groups, including for example, the formation of esters and ethers. One derivatization method involves the use of a carbodiimide for coupling to the surface.

Exemplary carbodiimides include, for example, dicyclohexylcarbodiimide (DCC), or (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) (EDC).

[00199] The redox active moieties can also be attached covalently to the surface by reactions with the native oxide, -O or -OH on the semiconductor surface. Many methods are known for carrying such reactions to form covalent bonds through various functional groups (see Maoz et al., *J. Colloid. Interface Sci.*, 1984, 100, 465-496). In some embodiments an indirect approach can be employed in which an alkoxysilane comprising other reactive functionality is reacted with the -O or -OH groups on the semiconductor surface to covalently attach the alkoxysilane to the semiconductor surface. The other reactive functionality on the alkoxysilane can then be used to covalently attach a redox active moiety to the surface. In these embodiments, the alkoxysilane can become a linker or portion of a linker. The other reactive functionality can be any reactive functionality that can be used to attach a redox active moiety. The functionality can be, for example, an olefin, an acetylene, an amine, a mercaptan, or an epoxy group. The reaction used to couple the alkoxysilane to the redox active moiety can be, for example a, Diels–Alder reaction, Michael addition, click chemistry, or epoxy chemistry. Semiconducting polymers comprising redox active moieties can be polymerized onto a surface, graft polymerized onto a surface, photo-polymerized, or pre-formed and cast onto a surface.

[00200] In some embodiments, the reactions described above for covalently attaching functional groups to the semiconductor surface are used to attach a linker group or portion of a linker group having a chemical

functionality that can be used to covalently bind the redox active moiety to the surface in a subsequent step.

[00201] Reactions that can be used to covalently attach a redox active moiety to the semiconductor surface include hydrosilylation, free radical reactions, carbodiimide coupling, Diels-Alder reactions, Michael addition, epoxy reactions, or click chemistry (see, e.g. Evans et al. *Australian Journal of Chemistry* 60 (6): 384-395 (2007)).

[00202] Organic semiconductor substrates of the invention can in some embodiments be modified to attach redox active species after the formation of the organic semiconductor. For example, an organic semiconductor can be cast into a film. In some embodiments the organic semiconductive polymers, for example, polyaniline, polypyrrole, and polythiophene are prepared by chemical or electrochemical oxidation. The semiconductive polymer can be subsequently modified using, for example, the chemistry described for the attachment of redox active agents to the inorganic semiconducting substrates. In some embodiments, the semiconductive polymers can comprise functional groups that can be used to attach redox active species to the semiconductive polymer. For example, the sulfonic acid groups in poly(anilinesulfonic acid) can be used to immobilize aminoferrocene, 1,1'-diaminoferrocene, and aminoanthracene through sulfamide formation. In some embodiments, a pyrrole, thiophene or aniline monomer can have a functional group such as an N-hydroxy succinimide ester, a carboxylic acid, or a reactive vinyl or allyl group. The monomers can then be polymerized, for example electropolymerized, and the redox active moiety can be attached to the functional group on the polymerized monomer.

[00203] In some embodiments, the redox active moiety can be incorporated into the semiconductive polymer itself, for example as part of the backbone of the polymer. For example, redox active groups within poly(ferrocenyl vinylene phenylene vinylene), poly(fluorine), and poly(carbazole) that comprise redox active moieties in their main chains, which can be used for analyte, e.g. pH sensing.

[00204] In some cases the surface of the semiconductor is modified by coating the surface with conductive compounds such as metals or metal oxides. In some embodiments, the semiconductor is coated with gold, silver, palladium, copper, platinum or other metals. The metals can be coated from solution, for example by electrodeposition, or can be coated onto the surface with vacuum techniques such as plasma deposition, or metal vaporization. The semiconductor surface can be coated with conductive or semiconductive metal oxide compounds such as indium-tin oxide. When these materials are coated onto the semiconductor electrode surface, the redox-active agents are attached to the semiconductor electrode by attachment to the layer on the semiconductor electrode.

[00205] Where a linker group is used, the linker can be small, for example one to 3 atoms, or can be longer, e.g. 20 to 100 atoms. The linker can also be any size between the small or longer linker. In some embodiments, the linker is relatively short allowing for the redox active moiety to be close to the surface, which can be beneficial for electron transfer. In some embodiments the linker is provided such that the redox active agent is held 1, 2, 3, 4, 5, 6, or 7 atoms from the surface of the semiconductor. Where a short linker is used, the redox-active moiety is held close to the surface. Where a longer group is used, the redox active moiety may be able to move away from the surface, for example further out into the

solution. Linker groups can comprise hydrophilic, hydrophobic groups, or mixtures thereof. Linker groups can comprise, for example, hydrocarbons, alkenes, alkynes, esters, ethers, amides, amines, carbonyls, thiols, olefins, silicones, or other organic, inorganic or organometallic groups. The linker groups can be formed by polymerization or oligomerization reactions such as free radical or anionic polymerization. The linker group can comprise, for example, ethylene oxide, propylene oxide, or acrylamide repeat units. Linkers can have ring structures including aromatic rings. The variation in the linker structure can be used to vary the mobility of the redox-active moiety in the solution. If the linker is too long and densely packed the redox active moiety can be far enough away from the surface such that the electron transfer to the electrode surface will be compromised. In these cases, having a linker that has electrical conductivity can be useful.

[00206] A redox-active moiety may be highly substituted, and can still act as a redox-active moiety. Thus, for example, the redox-active moiety ferrocene includes substituted ferrocenes, ferrocene polymers, and ferrocene covalently attached to the surface via linker molecules.

[00207] In some embodiments, the redox-active moiety can be incorporated into a polymer, and the polymer comprising the redox active moiety can be immobilized onto the semiconductor surface. The immobilization of the polymer can be either chemical or physical. The immobilization of the polymer can be through covalent bonds, or through adsorption of the polymer to the semiconductor surface.

[00208] The redox active moieties can be incorporated into any type of polymer that can be immobilized onto the surface of the semiconductor surface. Types of polymers that the redox active moieties can be incorporated into include biopolymers such as RNA, DNA or proteins, conductive polymers, fluoropolymers, polyterpenes, inorganic polymers, phenolic resins polyanhydrides, polyesters, polyolefins, polysiloxanes, polyamides, polyimides, polyethers, polyketones, polysulfones, and vinyl polymers.

[00209] The polymer comprising the redox active moieties can, in some cases, be produced at the semiconductor surface. For example monomers or oligomers comprising the redox active moieties can be polymerized in the region of the surface to product the polymer near the surface. In some cases, the polymerization can be initiated at the semiconductor surface, resulting in polymer covalently bound to the surface. The polymerization can be initiated at the surface can be initiated, for example by a free radical reaction initiated by a diazo group attached to the semiconductor surface. In other cases, the polymerization can be initiated in solution, for example near the surface, such that the nascent polymer is immobilized onto the surface as it is formed. Methods for determining the appropriate solvent conditions are known. For example by establishing that the monomer and/or oligomer are soluble, while the polymer is insoluble, allowing for surface deposition to occur. The semiconductor surface can comprise polymerizable functional groups that are capable of copolymerizing with the monomers or oligomers comprising the redox active moieties resulting in covalently binding the redox active polymer onto the semiconductor surface.

[00210] In some embodiments, polymer comprising the redox active moieties can be electropolymerized at the semiconductor surface. For example, monomers comprising the redox active moieties are added to

a solution, and current is provided through the semiconductor surface causing electropolymerization of the monomers. In some embodiments, the electropolymerization can result in the covalent attachment of the electropolymerized polymer to the semiconductor surface. In other embodiments, the electropolymerization can result in polymerization, for example, at the semiconductor-solution interface, and the polymer that is formed can deposit onto the semiconductor surface, resulting immobilization of the polymer by physisorption to the surface. The polymers can be polymerized onto a surface, graft polymerized onto a surface, photo-polymerized, or pre-formed and cast onto a surface.

[00211] The polymers can be chemically or electrochemically deposited on individual microelectrodes, polymerized, as respond to a signal in a reversible manner, in a way that can be electrochemically detected. Other such materials are described by R. W. Murray in *Electroanalytical Chemistry*, vol. 13, edited by A. J. Bard (Marcel Dekker, NY 1984), the teachings of which are specifically incorporated herein.

[00212] In some embodiments, the polymer is formed away from the semiconductor surface, and subsequently immobilized thereto. The polymer can be immobilized onto the surface by a variety of methods including, adsorption from solution, coating including spin coating and dip coating, spraying, printing, electropainting, or electrodeposition.

[00213] In some embodiments the semiconductor electrode is formed by contacting an H-terminated semiconductor surface with the one or more redox-active moieties wherein at least one redox active moiety is sensitive to the presence of an analyte, wherein each redox-active moiety comprises a functional group that will react with the H-terminated semiconductor surface to form a covalently bond, thereby forming a derivatized semiconductor surface. In some embodiments, the surface comprises at least two redox active moieties and one of the redox active moieties is insensitive to the presence of the analyte

Uses of the Compositions and Devices

[00214] One aspect of the invention is a method of determining the concentration of an analyte. In one embodiment, the method comprises: placing an electrode in contact with said analyte, said electrode comprising a semiconductor substrate with semiconductor surface having immobilized thereon an analyte-sensitive redox-active moiety, said analyte-sensitive redox-active moiety exhibiting an oxidation potential and/or reduction potential that is sensitive to the concentration of the analyte; applying a plurality of potentials to the electrode; and measuring the current through the electrode at the plurality of potential, determining a reduction and/or oxidation potential of the analyte-sensitive redox-active moiety, thereby determining the concentration of the analyte.

[00215] In one aspect the method comprises: determining the concentration of an analyte by (a) placing an electrode in contact with said analyte, said electrode comprising a semiconductor substrate with semiconductor surface having immobilized thereon an analyte-sensitive redox-active moiety, said analyte-sensitive redox-active moiety exhibiting an oxidation potential and/or reduction potential that is sensitive to the concentration of the analyte; (b) applying a plurality of potentials to the electrode; and (c) measuring the current through the electrode at the plurality of potentials, and determining a reduction

and/or oxidation potential of the analyte-sensitive redox-active moiety, thereby determining the concentration of the analyte.

[00216] The method of determining the concentration of the analyte can be used to measure pH by utilizing redox active moieties that are sensitive to hydrogen ion as described above.

[00217] The measurement of current at a plurality of potentials allows for carrying out voltammetry for determining the oxidation and or reduction potential of the redox active moiety or moieties immobilized on the surface. The voltammetry used in the method can be, for example cyclic voltammetry, pulse voltammetry, normal pulse voltammetry, square wave voltammetry, differential pulse voltammetry linear voltammetry, or square wave voltammetry. The source for supplying a plurality of potentials can be a potentiostat, for example, a potentiostat capable of applying square waves for square wave voltammetry.

[00218] The frequency of the measurement can affect the quality of the data. In some embodiments, square wave voltammograms are currently at a step height of 2 mV, amplitude of 25 mV and a frequency of 10 Hz. In some cases, it is advantageous to increase the frequency. For example, increasing the frequency to 500 Hz can result in a faster scan rate. We have observed that in some cases, a higher frequency results in a higher level of observed current. In some cases, the peak current can shift, for example to more negative potentials upon increasing the operating frequency. In some cases changing the potential at higher frequencies can result in more noise in the square wave voltammograms. In some cases it is advantageous to shield the electrode from the light because in some cases, light can contribute to the background noise.

[00219] The method often further comprises an analyte-insensitive redox-active moiety having a reduction and/or oxidation potential that is substantially insensitive to the analyte, further comprising determining the oxidation and/or reduction potential of the analyte-insensitive redox-active moiety, and determining the concentration of the analyte from the difference in the oxidation and/or reduction potentials of the analyte-sensitive and analyte-insensitive moieties.

[00220] Generally, the analyte concentration is determined by using voltammetry to identify the position of current peaks, which current peaks indicate the reduction or oxidation potential of a redox active moiety. In some embodiments, the position of the reduction and/or oxidation potential of the analyte sensitive redox active moiety is used to determine the concentration of the analyte. For example, the position of the current peak with respect to the potential at a reference electrode can be used. This method can be used, for example, where no analyte insensitive redox active moiety is employed.

[00221] Where an analyte insensitive redox active moiety is used, detection is generally accomplished by measuring the potential difference, delta E, associated with current peaks for oxidation (or reduction) of the immobilized redox active moieties, where the magnitude of delta E can be related to the concentration of analyte, e.g. hydrogen ion (H⁺) in solution. That is, in many embodiments, delta E represents the potential difference between the reduction and/or oxidation potential between a redox active analyte sensitive moiety and a redox active analyte insensitive redox active moiety. The analyte insensitive redox active moiety which has an electrochemical response that is insensitive to variations in the medium serves as the reference. Current peaks for oxidation or reduction of the reference and indicator can be

determined from a voltammograms using a counter electrode, and without the need for a reference electrode.

[00222] The method calls using the measured current through the electrode at the plurality of potentials to determine the concentration of the analyte. The determination of the concentration using the measured current (e.g. current peaks) can be accomplished by using a computation system that communicates with the device for measuring current. The computation system can apply algorithms for calculating reduction or oxidation potential from the measured current at a plurality of potentials from the voltammetry measurements. The computing systems can be part of the sensing system, in some cases allowing the sensing system to be self contained. The computing system can utilize its memory for storing raw or processed data from the sensors. The method can further comprise communication between the computing system and the sensor via transmission device that will wirelessly or by wire transmit processed data to an external device.

[00223] Carrying out the method typically requires the use of at least one other electrode (the counter electrode). The counter electrode is used to complete the electrochemical circuit to make the measurements described herein. The counter electrode is generally made of a material that is chemically inert to the medium so that its potential does not change significantly during the course of measurement. Suitable materials in many applications include platinum, gold, stainless steel, and carbon. In some cases, the counter electrode can be incorporated into the chip that also comprises the semiconductor sensor electrode.

[00224] A reference electrode is optional and is used as an additional electrode in some embodiments of the method of measuring analyte concentration. In the case of a three-electrode system, the counter electrode generally completes the circuit, allowing current to flow through the cell, while the reference electrode maintains a constant interfacial potential difference regardless of the current. In the case where the system comprises an analyte sensitive redox active moiety and an analyte insensitive redox active moiety, the analyte insensitive redox active moiety can act as a reference, allowing the potential difference to be used to determine analyte concentration. Even when an analyte insensitive moiety is also used, in some embodiments, a reference electrode will still be used. In some embodiments, pseudo-reference electrodes can also be utilized. Reference electrodes which can be employed are described above.

[00225]. In many embodiments, the sample is a liquid sample, and the electrodes are each in contact with the liquid. In some cases, the sample will not be a liquid, but will be a solid, generally comprising a solid electrolyte, or a gas.

[00226] In some embodiments, the method involves the in-line sensing of a process. An in-line sensor can be a sensor that is used in an on-going process. In some embodiments the method comprise the use of a sensor is in a vessel, in other embodiments the sensor is in a conduit or pipe through which a process fluid flows. In some embodiments, the method comprises using currents measured at a plurality of potentials by voltammetry to determine analyte concentration, and the determined analyte concentration is used to control a process parameter. The systems of the present invention are valuable in in-line sensing in that

they can be made to be robust, to resist fouling, and are able to measure analyte concentration for long periods of time in media that changes its properties, as in a process such as a chemical reaction, biochemical reaction, or fermentation.

[00227] One aspect of remote monitoring is that the sensor can be programmed to automatically take readings. The automatic readings can be programmed to occur on a periodic basis, to occur upon the happening of some event, or to occur when the sensor is prompted. The periodic events can be separated on the order of seconds to on the order of months. The happening of some event could be, for instance at the point when the measured solution reaches a certain volume level, or at given points in the steps of a manufacturing process (e.g. at the beginning of or end of a step, or upon the addition of a reagent to a vessel).

[00228] Remote monitoring generally includes communication from the remote sensing unit, and/or communication to the remote sensing unit. The communication to and from the remote sensing unit can be done with transmission lines, and/or wirelessly. Any type of signal including, for example, digital, analog, wideband, narrowband, or combinations thereof, can be used.

[00229] One aspect of the present invention is the voltammetric monitoring of pH with a semiconductor electrode as part of process control in processes. In one embodiment, a voltammetric pH measurement is made in an industrial process stream, and the pH value from that measurement is used to as input to a decision on the adjustment a process parameter. In one embodiment, the pH value from the voltammetric pH measurement with a semiconductor electrode is used to decide whether or not to add one or more components to the process, and/or to decide how much of the component to add. In some embodiments, the pH value is used to control the pH in a part of the process, for example, as input into the decision on the addition of either acidic or basic components. In some embodiments, the pH value is used to determine whether a process has reached a certain stage, for instance, whether a reaction is at completion. In some embodiments, the pH value is used to determine the addition of nutrients or other components to a reaction containing an organism to maintain the health and productivity of the organism.

[00230] The process control step can be automated such that a given pH measurement value from the sensor results in the change of a process parameter without the intervention of a person. In other embodiments, the pH measurement is viewed by a person who uses the information to make the decision the change of a process parameter.

[00231] The process control step can be controlled by a voltammetric pH system with a semiconductor electrode that has a sensor, a voltage source, a current measuring detector, and a computer for determining the pH from the current measurements. The voltammetric pH system can be in communication either with a process control system, or with an operator, by analog or digital means, either with a wire, wireless connections, fibers or combinations thereof.

[00232] One aspect of the invention is a method of voltammetric pH sensing with a semiconductor electrode wherein the pH sensor requires little calibration. In one aspect of the invention the pH sensor is substantially free of the need for calibration.

[00233] The use of the voltammetric pH sensing with a semiconductor electrode has a number of

advantages. For example, the sensors of the present invention generally comprise solid-state sensors. The sensors of the present invention have a built-in internal standard such that calibration is not required. The sensors of the present invention can be constructed to be physically robust, such that they are not
5 prone to breakage. The sensors of the present invention can be made to be relatively insensitive to fouling. The sensors of the invention can be constructed to be resistant to chemical sterilization such as exposure to ethylene oxide, UV stabilization, gamma irradiation, electron beam irradiation, and temperature treatment. The sensors of the invention can be constructed to be resistant to high humidity and high temperature treatment under pressure such as experienced in an autoclave.

10 [00234] The voltammetric pH sensing methods with a semiconductor electrodes comprise reactions carried out in stainless steel reactors, glass reactors (e.g. for product development), and disposable reactors (e.g. plastic reagent bags), for example reactors described by manufacturers such as Wave Biotech, Hyclone, Xcellerex, and Stedim.

[00235] One aspect of the invention involves methods of voltammetric pH sensing with a semiconductor
15 electrode for processing including chromatography and tangential flow ultrafiltration.

[00236] Another aspect of the invention is as a sensor in a remote monitoring system such as a drug (pharmaceutical agent) delivery system. Such a system is described, for example, in U.S. Patent Application 2003/0153900. The analyte monitoring system or monitoring and drug (pharmaceutical agent) delivery system can be partitioned into a disposable module, a reusable module and a personal
20 digital assistant (PDA) module. A PDA is typically a portable, e.g. handheld device that has computing and networking capability, and a user interface, with output, e.g. a display, and input, e.g. stylus, keyboard, and/or touchscreen capability. This configuration optimally distributes functionality among these three configurations to achieve certain advantages. However the invention is not limited to this configuration. For example, a one-unit disposable device including all electronics, microneedles,
25 chemistry, sensors, mechanics and user interface may be alternatively employed. Or, more relevantly, the design of the invention allows for any distribution of components between one or more system modules. For example, components may be partitioned among one or more system modules based on the overall system cost, user safety and/or performance concerns.

[00237] The disposable module contains those components that once used must be discarded to maintain
30 safety and accuracy. This module preferably includes any structural or mechanical elements to maintain integrity, sterility and/or an electromechanical interface to any reusable components. Therefore this system module can include, for example: microneedles, a microfluidic assembly, membrane, reagent chemistry and associate housing materials. The portion of a sensor which is in contact with a biological fluid, for example, may be part of the disposable module. This module can also include retaining
35 mechanisms for establishing and maintaining intimate contact with the body thereby providing mechanical retention of the analyte monitoring/drug (pharmaceutical agent) delivery system.

[00238] The reusable module generally contains those components that control, automate motion, measure the analyte concentration, alarm the user, transmit data to the PDA module. This module can also

include retaining mechanisms. Generally, this module includes: a microprocessor with associated circuitry (e.g., memory, supporting electronics and the like), sensing circuitry, including, for example, a voltage supply and current measuring device, drive mechanisms such as motors or the like, a power supply (e.g., battery) and an interface operable to communicate with a portable computing device or PDA. The interface can be RF, magnetic or inductive, optical or the like. The reusable module can also have an audible or vibration alarm to notify the user that user action intervention is required.

[00239] The PDA module generally includes a separate user interface via a portable computing device such as a personal digital assistant (PDA), handheld computer or the like for controlling and/or interacting with the device. A typical portable computing device includes a processor, memory, associated circuitry, a display (e.g., monochrome or color LCD) and an input device such as a key pad, touch screen (e.g., integrated with the display) or the like and an operating system. The display can show the value of the analyte to be measured, could provide the user with instructions on how to respond to the measured level of analyte, or may tell the user what automatic actions have been taken in response to a measured level of analyte.

[00240] Today, portable computing devices with improved operating system software and user interfaces are readily available. These devices provide the potential for rich and extended functionality. For example a typical PDA includes a relatively large viewing screen and can also include wireless communications mechanisms, a sophisticated operating system and a variety of business and personal software (calendars, scheduling, etc.). The invention preferably includes the use of a PDA to provide the proprietary software (programs) for autonomous operation with an improved user interface.

[00241] For example, the PDA module can provide the user with software that facilitates informed decisions to help a patient user more optimally adjust either drug levels or behaviors to more optimal levels. The PDA configuration provides a user interface and preferably allows users the ability to program and or control testing. The user can view individual analyte measurements and graphically display analyte level trends by the day, week or custom time period. The PDA can be used to display any and all of the measurements recorded by the system. Using the proper software, the user can be provided with recommendations for drug regiment modification. In some cases, the user can program the times when their analyte tests are to be taken. Preferably, the user can also set the upper and lower limits for alerts.

[00242] The system can be programmed such that whenever a user makes changes and with verification from the user, the information can be wirelessly downloaded to the system. During the day the user may not need to use the PDA unless alerted by the system to check for an analyte reading. The user can initiate a test from the PDA if wanting to make an immediate measurement. Once the user selects this command, verifies it, and transmits it to the reusable module, a confirmation is made back to the PDA.

[00243] In one aspect the invention comprises drug dispenser capsule comprising a voltammetric sensor. In some embodiments, the drug dispenser capsule comprises a semiconductor based voltammetric sensor as described herein. The drug dispenser capsule of the present invention internally senses a biologic condition by the detection of the presence or amount of an analyte, and internally dispenses drugs within the digestive tract of a body (e.g., a human body or animal body) based upon the sensed level of analyte.

The capsule is inert and is therefore swallowable and passable through the digestive tract without being consumed. By sensing the level of one or more analytes, the swallowable drug dispenser capsule senses information the digestive tract or senses conditions within the digestive tract that are indicative of conditions in other organs (e.g., skin). In addition to the voltammetric analyte sensor, the capsule contains one or more other sensors (e.g. chemical, electrical, etc.) so that more types of biologic data can be tracked through the digestive system. In response to that sensed information, the capsule dispenses a bioactive substance within the digestive tract without the need to transmit or receive signals from a remote transmitter/receiver, and without active human or computer management. Drug dispenser capsules are described, for example, in U.S. Patent 6,929,636.

[00244] The swallowable drug dispensing capsule comprising a voltammetric sensor can include, for example, sensors, a controller, memory, optional programmable logic, a power supply, a microactuator, a drug storage module, and communication interface having at least one of the following types of communication modules: radiofrequency; ultrasonic; and/or infrared. In one preferred embodiment, at least memory, and preferably also controller and/or programmable logic are embodied on a semiconductor-based, e.g. silicon-based, module in one or more semiconductor chips.

[00245] In some embodiments, the swallowable drug dispensing capsule has multiple sensors that are arranged an outer surface of capsule in a desired predetermined orientation that is expected to expose each sensor to a targeted bodily condition or landmark within the human body. Each sensor can comprise a single type of sensor such as an image detector or a different type of sensor (e.g. chemical, electrical, temperature, etc.). Chemical detectors detect the presence of many analytes, such as pH, or other analytes.

[00246] The swallowable drug dispensing capsule of the invention can have a controller that regulates communication between sensors and memory, communication between memory and any remote controllers outside of the human body, and communication with programmable logic component(s). Finally, controller can operably control both communication interface and a microactuator. The controller typically is a logic controller and includes a microprocessor. The controller may also comprise one or more logical devices (e.g., a logic gate) capable of performing a sequence of logical operations.

[00247] The swallowable drug dispensing capsule generally has a memory or storage device that is typically an ultra-high capacity storage device, and which is often based on a semiconductor chip, e.g. a silicon chip.

[00248] The swallowable drug dispensing capsule generally has a drug storage module and a microactuator. The drug storage module represents a container for holding a drug or bioactive substance that will be released, for example, into the digestive tract. Accordingly, the drug storage module also includes one or more selectively activated dispensing ports that open in an outer surface of capsule. The microactuator can have a chemically activated or electromechanically activated mechanism for causing the drug storage module to release its contents. The swallowable drug dispensing capsule has a suitable power supply, such as a lithium-ion battery, which is relatively non-toxic. Alternatively, other power supplies that are suitable for in vivo environments can be used.

[00249] The swallowable drug dispensing capsule generally has a communication interface that includes any suitable wireless transmission technology (e.g. ultrasonic, radiofrequency, etc.) that readily permits communication to and from the capsule while the capsule is in digestive tract and the remote transmitter/receiver which is located remotely outside of the body. However, a wireless port is preferably used for communicating with capsule after capsule is captured from the body. Likewise, a wireless port may be used for programming the controller, memory, and/or logic component prior to insertion of capsule within the body to determine the manner that the sensors will operate and communicate with the memory, as well as the manner that microactuator will operate and communicate with memory via controller.

[00250] In use, the sensors, including the voltammetric sensor of the capsule sense analyte concentrations and biologic data within the digestive tract and the sensed data is passed through the controller for storage in memory and/or comparison with a stored data profile in memory and/or logic. After the predetermined criteria are met, controller activates microactuator to dispense the drug from drug storage module into digestive tract. The sensed data optionally is stored in memory and retrieved via the communication interface after capture of capsule upon exiting the digestive tract. Finally, a wireless communication system optionally can be used in addition to, or as an alternative to, controller and memory to facilitate evaluating and storing sensed data and to dispense drugs upon selective activation at the appropriate time.

[00251] The semiconductor electrode voltammetric pH sensors of the present invention can be used in manufacturing operations such as the manufacture of coatings, cleaners and sealers that enhance paint and finish bonding, metal passivation to protect substrates during shipment and storage, paint spray booth treatments that enhance quality and efficiency, and air scrubbers that limit pollutant emissions. In these applications, the reliable measurement of pH can be an integral part of the process.

[00252] In one aspect the sensors of the present invention can be used as embeddable corrosion measuring instrument that is capable of providing information related to corrosion rate, corrosion potential, conductivity and chloride concentration, and pH levels of steel rebar reinforced structures. The devices can be used to monitor the integrity of the steel. The devices and systems of the present invention do not require a direct electrical connection to the reinforcement steel within the structure, using the structural steel as one of the referencing materials. Since the disclosed instruments do not require proximity to the steel within the structure, the instruments can be dispersed at critical locations within the structure, regardless of steel placement. In some embodiments, the systems and devices are self-contained, incorporating all required sensing electrodes and electronics. The devices can be deployed as described in U. S. Patent 6,690,182.

[00253] The semiconductor electrode voltammetric pH sensors of the present invention can be used in winemaking. Measurements of various properties including pH are taken throughout the process, including during (1) pressing, (2) primary fermentation, which often takes between one and two weeks, where yeast converts most of the sugars in the grape juice into ethanol (alcohol) (3) secondary fermentation.

[00254] The semiconductor electrode voltammetric pH sensors of the present invention can be used in brewing. The measurement of pH can be important at the various stages of brewing, for example, at mashing, lautering, lauter tun, mash filter, boiling, whirlpool, wort cooling, fermenting, conditioning, filtering, and secondary fermentation. The semiconductor electrode voltammetric pH sensors of the present invention can be particularly important during fermentation, where the voltammetric pH sensors of the present invention are advantageous as they require little to no calibration, and can be made to resist fouling during the fermentation process.

[00255] The semiconductor electrode voltammetric pH sensors of the present invention can be used in the production of biofuels, including the production of biodiesel, ethanol, butanol, and substitutes for gasoline, diesel, jet fuel, and additives to be used in any of the foregoing. The production of ethanol includes both the process of converting the cellulose to sugars, and the process of converting the sugars to ethanol. Although there are several key technological differences in how ethanol is produced from corn or cellulosic feedstock, both paths to ethanol production typically require a fermentation step that involves the conversion of glucose and other sugars to ethanol. Currently, baker's yeast, *Saccharomyces cerevisiae*, provides the primary microbiological system used by the corn-based ethanol industry. The methods of the present invention relate to ethanol production for fuel from *Saccharomyces cerevisiae* and other organisms. The control of pH can be useful in catalytic biofuel production processes, such as for the production of biofuel.

[00256] The semiconductor electrode voltammetric pH sensors of the present invention can be used in oil recovery and refining. The sensors can be incorporated into down-hole devices for measuring the analytes present in the down-hole environment. The sensors can be used in other aspects of processing the oil such as in oil refining.

[00257] The semiconductor electrode voltammetric pH sensors of the present invention can be used in the production of biopharmaceuticals, for example, medical drugs produced using biotechnology. They include, for example, proteins (including antibodies), nucleic acids (DNA, RNA or antisense oligonucleotides) used for therapeutic or in vivo diagnostic purposes. Biopharmaceuticals are produced by means other than direct extraction from a native (non-engineered) biological source. An example is recombinant human insulin (rHI, trade name Humulin), which was developed by Genentech and marketed by Eli Lilly.

[00258] An aspect of the invention is a semiconductor electrode voltammetric pH sensor for the production of biopharmaceuticals including: blood factors (e.g. Factor VIII and Factor IX), thrombolytic agents (e.g. tissue plasminogen activator), hormones (e.g. insulin, growth hormone, gonadotrophins), haematopoietic growth factors (e.g. erythropoietin, colony stimulating factors), interferons (e.g. interferons- α , - β , - δ), interleukin-based products (e.g. interleukin-2), vaccines (Hepatitis B surface antigen), monoclonal antibodies (e.g. infliximab, basiliximab, abciximab, daclizumab, gemtuzumab, alemtuzumab, rituximab, palivizumab, trastuzumab (herceptin), and etanercept) and other products such as tumor necrosis factor, and therapeutic enzymes.

[00259] One aspect of the invention is a method of making a protein comprising carrying out a

fermentation reaction that produces such protein wherein the pH of the fermentation is controlled by measuring the pH with a pH sensor comprising a semiconductor surface having immobilized thereon a redox active moiety that is sensitive to the presence of hydrogen ion, and using the measured pH to control the pH of the fermentation reaction. In some embodiments, the control of the pH can be manual, for instance, where an operator reads the pH from the pH sensor and uses the measured pH to determine whether or how much to adjust the pH, and in other embodiments, the control can be automatic, where the pH measurement is read by instruments that can adjust the pH based on the value of the measurement received.

[00260] The semiconductor electrode voltammetric pH sensors of the present invention can be used for biopharmaceuticals produced from microbial cells (e.g. recombinant E. coli), mammalian cell lines and plant cell cultures in bioreactors of various configurations.

[00261] Cell culture requires cells to be grown, often under a strict set of conditions to maintain the health of the cells and maximize the production of the culture. Cells are grown and maintained at an appropriate temperature and gas mixture (for example, 37°C, 5% CO₂) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

[00262] Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrient components. The effect of changes in pH can be dramatic in some cases, and it can be important to maintain the pH. The devices, systems, and methods of the invention allow for control of pH within a range of 1, 0.5, .02, 0.1, 0.05, 0.02, 0.01 pH units or less to maintain the growth and health of the cells. The semiconductor electrodes of the present invention allow for the accurate measurement of pH with limited fouling, and in some embodiments, no need for calibration.

[00263] The semiconductor electrode voltammetric pH sensors of the present invention can be used for cells that are grown completely in solution, and for cells that are grown on a substrate. Some cells naturally live without attaching to a surface, such as cells that exist in the bloodstream. Others require a surface, such as most cells derived from solid tissues. Cells grown unattached to a surface are referred to as suspension cultures. Other adherent cultures cells can be grown on tissue culture plastic, which may be coated with extracellular matrix components (e.g. collagen or fibronectin) to increase its adhesion properties and provide other signals needed for growth.

[00264] One aspect of the invention is a bioreactor or fermentor in which the reaction or fermentation occurring therein is controlled by a semiconductor based voltammetric sensor of the invention. In one embodiment, the invention comprises a bioreactor comprising a semiconductor based sensor wherein the semiconductor sensor comprises a semiconductor surface having immobilized thereon a redox active moiety that is sensitive to the presence of an analyte, such as hydrogen ion. Figure 17 shows an example of a bioreactor of the invention comprising a probe for measuring pH, and thereby controlling the pH in the reactor during the reaction. The probe comprises an electrode having a semiconductor surface having

immobilized thereon a redox active moiety that is sensitive to hydrogen ion. In some embodiments the probe comprises two electrodes, each comprising a semiconductor surface, one of the electrodes having immobilized thereto a redox active moiety that is sensitive to hydrogen ion, and one of the electrodes having attached thereto a redox active moiety that is insensitive to hydrogen ion. In some embodiments the probe further comprises a counter electrode, and in some embodiments it further comprises a reference electrode.

[00265] The semiconductor electrode voltammetric pH sensors of the present invention can be used to assist in the successful manipulation of cultured cells. As cells generally continue to divide in culture, they generally grow to fill the available area or volume. This can generate several issues that the reliable measurement of pH can assist with, such as: Nutrient depletion in the growth media; accumulation of apoptotic/necrotic (dead) cells; cell-to-cell contact stimulating cell cycle arrest, causing cells to stop dividing known as contact inhibition; cell-to-cell contact stimulating promiscuous and unwanted cellular differentiation. Sometimes these issues can be identified by monitoring pH, alone or in combination with other measurements, and can then be controlled or remediated by adjusting tissue culture conditions that often rely on sterile techniques. These methods aim to avoid contamination with bacteria or yeast that will compete with mammalian cells for nutrients and/or cause cell infection and cell death. The pH measurements of the present invention are amenable to being carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms.

[00266] The semiconductor electrode voltammetric pH sensors of the present invention can be used for pH sensing in plant tissue culture, bacterial and yeast cell culture, and viral cell culture.

[00267] An aspect of the invention is a semiconductor electrode voltammetric pH sensor for sensing pH in plant tissue culture. The pH measurements of the present invention can be used at any step of plant cell culture. Plant tissue culture is typically performed under aseptic conditions under filtered air. Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions (usually alcohol and mercuric chloride) is an important first step. Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Solid and liquid media are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones. Solid media are prepared from liquid media with the addition of a gelling agent, usually purified agar. The pH measurements of the present invention can be made in the liquid, in the moist soil, or in the agar. The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids), and the pH, can have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots.

[00268] The semiconductor electrode voltammetric pH sensors of the present invention can be used with any cell line including: National Cancer Institute's cancer cell lines, zebrafish ZF4 and AB9 cells, Madin-Darby Canine Kidney MDCK epithelial cell line, Chinese Hamster Ovary CHO cells, Insect cell line

Sf21, MCF-7 (breast cancer), MDA-MB-438 (breast cancer), U87 (glioblastoma), A172 (glioma), HeLa (cervical cancer), HL60 (promyelocytic leukemia), A549 (lung cancer), HEK 293 cells (kidney - original HEK line is contaminated with HeLa), SHSY5Y Human neuroblastoma cells, cloned from a myeloma, Jurkat cell line, derived from a patient with T cell leukemia, BCP-1 cells (PEL), Primate cell lines, Vero (African green monkey *Chlorocebus* kidney epithelial cell line initiated 1962), COS-7 (African Green Monkey Kidney Cells), Rat tumor cell lines, GH3 (pituitary tumor), 9L (glioblastoma), Mouse cell lines, 3T3 cells, MC3T3 (embryonic calvarial), C3H-10T1/2 (embryonic mesenchymal), NIH-3T3 (embryonic fibroblast), Invertebrate cell lines, C6/36 *Aedes albopictus* (Asian tiger mosquito) larva, Plant cell lines, Tobacco BY-2 cells (kept as cell suspension culture, they are model system of plant cell).

[00269] An aspect of the invention is a semiconductor electrode voltammetric pH sensor for use in water purification. Water purification is the process of removing contaminants from a raw water source, the goal is generally to produce water for a specific purpose with a treatment profile designed to limit the inclusion of specific materials. Water purification is not only water purified for human consumption or drinking water. The semiconductor electrode voltammetric pH sensors of the present invention can also be used water purified to meet the requirements of medical, pharmacology, chemical and industrial applications. The semiconductor electrode voltammetric pH sensors of the present invention can be used in water purification processes including, but not limited to ultraviolet light; filtration; water softening; reverse osmosis, ultrafiltration; molecular stripping; deionization; and carbon treatment. Water purification may remove particulate sand, suspended particles of organic material; parasites, such as giardia; cryptosporidium; bacteria; algae; virus; fungi, etc; minerals such as calcium, silica, magnesium; and toxic metals such as lead, copper; and chrome. Some purification may be elective in its inclusion in the purification process; examples, smell (hydrogen sulfide remediation), taste (mineral extraction), and appearance (iron encapsulation).

[00270] Water from any source is applicable to the present invention. Groundwater (well water) is an economical choice for drinking water, as it is inherently pre-filtered, by the aquifer from which it is extracted. Water from an aquifer will have a limited output and can take thousands of years to recharge. Surface water; (rivers, lakes, streams) is far more abundant and is the typical raw water source used to make drinking water, as a water source it is carefully monitored for the presence of a variety of contaminants. The methods of the present invention encompass the voltammetric measurement of pH of these types of water where the pH value of the measurement can be used to decide on the purity of the water.

[00271] The semiconductor electrode voltammetric pH sensors of the present invention can be used with water purification methods including: pumping and containment, screening, storage, pre-conditioning, pre-chlorination, and removal of the fine solids, micro-organisms and some dissolved inorganic and organic materials.

[00272] Distilled water generally has an average pH of about 7 (neither alkaline or acidic) and seawater generally has an average pH of 8.3 (slightly alkaline). If the water is acidic (lower than 7), lime or soda ash can be added to raise the pH. Lime is the more common of the two additives because it is cheaper,

but it also adds to the resulting water hardness. Neutralizing with soda ash, however, increases the sodium content of the water. Making the water slightly alkaline helps ensure that coagulation and flocculation processes work effectively and also helps to minimize the risk of corrosion in pipes and pipe fittings. The pH value can be used to determine whether water is likely to be hard or soft. In general, water with a low pH (< 6.5) is acidic, and tends to be soft, and corrosive. Therefore, the water could contain metal ions, such as iron, manganese, copper, lead, and zinc. In some cases this results in elevated levels of toxic metals. This can cause premature damage to metal piping, and have associated aesthetic problems such as a metallic or sour taste, staining of laundry, and the characteristic "blue-green" staining of sinks and drains. More importantly, there are health risks associated with these ions or contaminants. The primary way to treat the problem of low pH water is with the use of a neutralizer. The neutralizer feeds a basic solution into the water to prevent the water from reacting with the household plumbing or contributing to electrolytic corrosion. Water with a pH > 8.5 could indicate that the water is hard. Hard water does not pose a health risk, but can cause aesthetic problems. These problems include an alkali taste to the water, formation of a deposit on dishes, utensils, and laundry basins, difficulty in getting soaps and detergents to lather, and formation of insoluble precipitates on clothing.

[00273] Another aspect of the invention is sensing of analyte levels such as the pH of bodies of water for example for resource control. The body of water can be, for example, a lake, ocean, stream, or river. The ability of the invention to be used remotely and to be used without the need for frequent calibration or any calibration at all allows the systems, devices, and electrodes to be deployed remotely in bodies of water to provide information on analytes such as hydrogen ion, etc. in such remote bodies.

[00274] An aspect of the invention is a semiconductor electrode voltammetric pH sensor for the measurement of pH in processes related to sewage treatment. Sewage treatment can have the same steps as described above, but may refer to water that has a higher level of contamination. Raw influent (sewage) can be the liquid and semi-solid waste from toilets, baths, showers, kitchens, sinks etc.

Household waste that is disposed of via sewers can compose the sewage. In some areas sewage also includes some liquid waste from industry and commerce.

[00275] The pH sensors of the present invention are suitable for single use, or for disposable applications.

[00276] The pH sensors of the present invention are amenable to miniaturization.

Examples

Reagents and Instrumentations

[00277] Vinylferrocene, vinylanthracene, hydrofluoric acid were purchased from Sigma-Aldrich (Sigma-Aldrich INC., USA), ferrocenecarboxaldehyde and mesitylene were obtained from Alfa Aesar (Alfa Aesar INC., USA), and 9-anthracene-carboxaldehyde was obtained from Acros Organics (Acros Organics INC. USA). All the chemicals were obtained with the highest grade available and were used without further purification.

[00278] Different single-side polished, primary flat, 500 μm thick silicon wafers with (111) and (100) orientation were purchased from Virginia Semiconductor with the following specification: i) P-type (100,

10-90 Ω -cm resistivity), ii) P-type (100, 0.001-0.005 Ω cm resistivity), iii) N-type (100, 10-40 Ω -cm resistivity), iv) N-type (100, 0.02-0.05 Ω -cm resistivity), v) P-type (111, 0.001-0.004- Ω cm resistivity) and vi) N-type (111, 0.001-0.005 Ω -cm resistivity).

[00279] Electrochemical measurements were recorded using an Autolab computer controlled potentiostat (Ecochemie, Utrecht, Netherlands) with a standard three-electrode configuration, consisting of a saturated calomel reference electrode (SCE, Radiometer, Copenhagen, Denmark), a platinum auxiliary electrode (Bioanalytical Systems INC., USA) and silicon (Virginia Semiconductor INC, USA) working electrode.

[00280] Different pH solutions in the range of 1 to 12 were also prepared in deionized water as follows:

pH 1.2, 0.10 M perchloric acid; pH 2.2, 0.05 M perchloric acid; pH 4.6, 0.1 M acetic acid + 0.10 M sodium acetate; pH 5.6, 0.5 M sodium acetate; pH 6.5, 0.025 M K_2PO_4 + 0.025 M KH_2PO_4 ; pH 7.33, 0.05 M K_2PO_4 ; pH 9.3, 0.10 M sodium borate; pH 13.5, 0.1 M sodium hydroxide. These solutions also contained an addition of 0.10 M sodium perchlorate as supporting electrolyte. The pH of these solutions was measured using the SevinMulti (Mettler Toledo) pH meter.

Example 1: Preparation of H-terminated silicon surface

[00281] Silicon wafers (oriented (111) or (100), cut into *ca.* 1x1 cm² pieces) were cleaned using "Piranha" solution (concentrated H_2SO_4 :30% H_2O_2 , 3:1, v/v) at about 80°C for 30 min and rinsed thoroughly with deionized water. (In some cases, smaller pieces, e.g. 2mm x 3.3 mm or 2mm x 2.7 mm were used.) Subsequently, the wafer pieces were oxidized in H_2O_2 :HCl: H_2O (2:1:8) at about 80°C for 15 min, and in H_2O_2 : NH_4OH : H_2O (2:1:8) at about 80°C for another 15 min, rinsed copiously with deionized water. The cleaned Si(100) wafers pieces were then etched in 2.5% HF solution for about 15 min. These procedures eliminate the native silicon oxide layer, yielding an H-terminated surface. The H-terminated substrates were quickly rinsed with deionized water, dried with nitrogen gas and were used immediately for the derivatization experiments. The Si(100) (10-90 Ω -cm, P-type) was used for the experiments below.

Example 2: Derivatization of H-terminated silicon surface with ferrocene moieties

[00282] Approximately 10 mmol mesitylene solution of vinylferrocene (VFc) or ferrocenecarboxaldehyde (FcA) was put in a round bottom flask and bubbled with nitrogen or argon gas for at least 30 min. A piece of the H-terminated silicon substrate was then immersed in the solution and allowed to react with VFc or FcA for about 12 h under reflux at about 150°C in an oil bath. During the reaction, the solution was also purged with nitrogen (or argon) to eliminate dissolved oxygen and to prevent the substrate from being oxidized. After the reaction, the substrate derivatized with VFc or FcA was rinsed with dichloromethane, acetonitrile, and methanol; and dried under a stream of nitrogen gas. The derivatization of the H-terminated surface with ferrocene moieties as described in Example 2 is illustrated in Figure 5.

Example 3: Derivatization of H-terminated silicon surface with anthracene moieties

[00283] Approximately 10 mmol mesitylene solution of vinylanthracene (VA) or anthraldehyde (AnA) was put in a round bottom flask and bubbled with nitrogen or argon gas for at least 30 min. A piece of

the H-terminated silicon substrate was then immersed in the solution and allowed to react with VA or AnA for about 12 h under reflux at about 150°C in an oil bath. During the reaction, the solution was also purged with nitrogen (or argon) to eliminate dissolved oxygen and to prevent the substrate from being oxidized. After the reaction, the substrate derivatized with VA or AnA was rinsed with dichloromethane, acetonitrile and methanol; and dried under a stream of nitrogen gas. The derivatization of the H-terminated surface with anthracene moieties as described in Example 3 is illustrated in Figure 6.

Example 4: Derivatization of H-terminated silicon surface with both the anthracene and ferrocene moieties

[00284] A 10 mmol mesitylene solution of anthracene (VA or AnA) and ferrocene (VFc or FcA) in 1:1 ratio was put in a round bottom flask and bubbled with nitrogen or argon gas for at least 30 min. A piece of the H-terminated silicon substrate was then immersed in the solution and allowed to react with the anthracene and ferrocene mixtures for about 12 h under reflux at 150°C in oil bath. During the reaction, the solution was also purged with nitrogen (or argon) to eliminate dissolved oxygen and to prevent the substrate from being oxidized. After the reaction, the derivatized substrate was rinsed with dichloromethane, acetonitrile and methanol, and dried under a stream of nitrogen gas. Figure 7 illustrates a reaction in which the silicon surface derivatized with both the anthracene and ferrocene using VFc and VA.

Example 5: Electrochemical measurements of the derivatized silicon wafers in different pH solutions

[00285] Square wave voltammetry (SWV) was carried out for the derivatized silicon wafers in a specially designed electrochemical cell as shown in Figure 8. The electrochemical measurements were performed using a standard three-electrode configuration. In these experiments, the derivatized silicon wafers were used as the working electrode, and was exposed to different pH solutions (about 10 mL) in the electrochemical cell. SWV were performed with a frequency of 10 Hz, a step potential of 2 mV and an amplitude of 25 mV.

[00286] The amperometric response of the anthracene or ferrocene derivatized silicon substrate at different pH solutions was studied using SWV. SWV was used as the electrochemical probe of the system because it produces a well-defined voltammetric peak in a single sweep. The corresponding square wave voltammograms recorded using a derivatized ferrocene electrode at different pH solutions from pH 1.23 to 9.33 are shown in Figure 9. These voltammograms show that as the pH values increase, the peak potential of the ferrocene peaks remain at the same peak potential. These results show that ferrocene is a pH insensitive molecule which can act as an internal reference material.

[00287] The corresponding SW voltammograms recorded using a derivatized anthracene electrode at different pH solutions, from pH 1.23 to 13.63, are shown in Figure 10 (a). These voltammograms show that as the pH value increases, the peak potential attributed to the anthracene shifts to a more negative potential. The corresponding plot of the peak potential against different pH is given in Figure 10(b). The

plot reveals a linear response from pH 1 to pH 14 with a corresponding gradient of ca 55.1 mV per pH unit. The ability of anthracene compounds to act as pH sensitive molecule is thus demonstrated.

[00288] The corresponding SW voltammograms recorded using a derivatized ferrocene + anthracene electrode at different pH solutions, from pH 1.23 to 9.33, are shown in Figure 11 (a). These voltammograms illustrate that as the pH values increase, the ferrocene peak remains at the same peak potential while the anthracene peak shifts to a more negative potential. The corresponding plot of the difference between the two peak potentials versus pH is shown in Figure 11(b). The plot reveals a linear response from pH 1 to pH 9.33 with a corresponding gradient of ca 45.1 mV per pH unit.

Example 6: Heat stability

[00289] A SW voltammogram was recorded for the silicon wafer derivatized with both VA and FcA moieties at room temperature in pH 6.52 buffer. The derivatized silicon sample was then autoclaved in a Consolidated Stills & Sterilizers for 40 min, and a SW voltammogram was recorded in pH 6.52 buffer after the autoclave. Next, the same sample was autoclaved again under the same condition 10 times, with a SW voltammogram recorded in pH 6.52 buffer after each autoclave.

[00290] The heat stability testing of the pH sensor was performed using a FcA+VA derivatized silicon sample. The resultant voltammograms are shown in Figure 12. A decrease in both the ferrocene and anthracene currents were observed after the first autoclave. Thereafter, both the peak currents remain relatively stable though subsequent cycles of autoclaving; in fact the peaks remain stable for ten cycles, showing that the sensor can withstand repeated heat sterilization.

Example 7: Fouling test

[00291] Four SW voltammograms were recorded for the four separate silicon wafers derivatized with FcA and AnA moieties at room temperature in pH 6.52 buffer. These derivatized samples were then autoclaved in the Consolidated Stills & Sterilizers for 20 min and were immersed in a 5 mL cell culture fermentation medium for six days. Then these samples were taken out of the cell culture medium, and SW voltammograms were recorded in pH 6.52 buffer again. SW voltammetry was also carried out in the cell culture medium, using the same silicon wafers.

[00292] The fouling testing of the pH sensor was performed using five derivatized silicon samples. The resultant voltammograms were shown in Figure 13. In all cases, the anthracene peaks remain stable with after six days exposure. The ferrocene peaks decrease after exposure, but the ferrocene peaks are still identifiable. These findings demonstrate that the pH sensors are still in good working order after six days exposure to the cell culture environment, demonstrating that the ability of the derivatized sensor to resist fouling. A control sensor was incubated for 6 days in culture fluid without cells or secreted proteins. This sensor's voltammogram exhibited a similar profile to the four that had been incubated in the actual cell fermentation environment, (Figure 14) suggesting that any loss of signal amplitude was not a function of cellular debris deposition.

Example 8: Stability of the Anthracene and Ferrocene derivatized Si surfaces

[00293] The stability testing of the Ac+Fc derivatized silicon surface was conducted for 22 days under continuous electrochemical measurement using PGSTAT12 autolab potentiostat in pH 4.65 buffer medium, scanning from -0.8 V to 0.8 V vs. Ag. The Ac peak remained as a well-defined peak with a full width half maximum (FWHM) of ~ 60 mV throughout the course of the experiments, while the Fc peaks broadened with time. Although the Fc peaks became broader, they are still identifiable and can be used as a reference. These findings demonstrate that the two-component derivatized silicon surface are still in good working order after 22 days continuous operation in pH 4.65 buffer medium, demonstrating the long term stability of the derivatized surface in the buffer medium.

[00294] In general, the Fc peaks are most well-defined and stable when the Fc molecules were derivatized onto a heavily doped silicon substrate. Figure 15(a) depicts SW voltammetric responses FcA on Si(100, N-type, 1-5 mΩ cm) in pH 7.33 buffer medium, showing every 50th scan of the 2,500 consecutive runs.; Figure 15(b) depicts voltammetric responses of VFc on Si(111, N-type, 0.02-0.05 Ω cm in pH 7.33 buffer medium, showing every 50th scan of the 2,500 consecutive runs. In some cases, both VFc and FcA moieties behave better on an N-type substrate than a P-type substrate in terms of the size of the peak current produced. The Fc derivatized silicon surfaces were all pH insensitive, *i.e.* the Fc peak does not shift upon exposure to different pH environments.

Example 9: Temperature Variation

[00295] The Nernst equation provides a theoretical framework for evaluating the temperature dependence of redox active species. It predicts that the slope of the peak potential against pH plot will increase as the temperature increases:

$$E_p = E_f^0 - \frac{2.3RTm}{nF} pH \quad \text{Eq. 1}$$

[00296] Where E_p is the peak potential (V), E_f^0 is the standard electrode potential (V), R is the universal gas constant (J K⁻¹ mol⁻¹), T is the absolute temperature (K), F is the Faraday constant (C mol⁻¹), m and n are the number of protons and electrons involved in the redox reaction, respectively. In the case of anthracene, the redox process for such molecules in the aqueous solution involves the participation of 2 electrons and 2 protons, thus $m=n=2$.

[00297] SW voltammograms were recorded for three pH solutions at pH 4.65, 7.33 and 9.35. Figure 16 shows the overlaid SW voltammograms of Ac derivatized silicon over the temperature range of 8 to 56 °C in pH 7.33 buffer medium. Similar responses were obtained at pH 4.65 and pH 9.35. There is a shift of the peak potential to more negative values with increasing temperature which may be attributed in part to a combination of changes in the reference couple, the temperature dependence of the formal potential (E_f^0), and the temperature term in Eq. 1. Analysis of the slope of the peak potential as a function of pH obtained experimentally at each temperature is tabulated in Table 1, which illustrates that the slope of pH against peak potential plot varies with temperature. The theoretical slope as predicted by the Nernst

equation as seen in *Eq 1* is also listed in Table 1 for comparison. As can be seen, the variation of the gradient of peak potential with pH is not Nernstian and indeed is relatively insensitive of temperature varying by about 2mV/pH unit over a temperature range of ~50°C. This dependence can be compared to a 10mV/pH shift that can be observed for a glass electrode. This small shift with temperature is beneficial in that it not only demonstrates that these Ac derivatized silicon wafers may be used as pH sensors at elevated temperatures, but also that they are not greatly affected by changes in temperature.

[00298] Table 1: A comparison of the theoretically predicted slope and experimentally obtained slope of the plot of pH against Ac peak potential for a range of temperatures.

T (°C)	8	17	28	44	56
T (K)	281	290	301	317	329
Theoretical (mV/pH)	55.7	57.5	59.7	62.8	65.2
Experimental (mV/pH)	55.0	55.5	56	56.3	57.0

Example 10: Stability during active measurement in cell culture medium

[00299] A fouling testing was carried out using anthracene derivatized silicon (100) wafer in the incubator (under controlled temperature of 37°C and CO₂ content of 5%) with continuous electrochemical measurements in cell culture (LP VA) medium for 7 days using a three-electrode setup connected to an Autolab type III potentiostat. The three-electrode setup (in an electrochemical cell) was autoclaved in a Consolidated Stills & Sterilizers autoclave for 40 min prior to adding 5 mL of the cell culture medium to the autoclaved setup. The electrochemical cell containing the medium was then transferred to the incubator, where continuous electrochemical measurements were performed.

[00300] Voltammograms were taken repeatedly over 7 days resulting in 10,000 consecutive voltammograms. Figure 18(a) shows voltammograms taken over the 7 day period (every 250th scan of the 10,000 consecutive runs). The anthracene peak was observed at ~-0.71V vs. Ag and remained consistent throughout the 7 days of in-situ electrochemical measurements in the cell culture medium. A plot of the anthracene peak potential over the 7 day time period is shown in Figure 18(b). These findings demonstrate that the anthracene derivatized silicon is still in good working order and that the peak potential remains consistent while the sensor is actively sensing the solution for 7 days.

Example 11: Derivatization of H-terminated silicon surfaces with 2-allyl-1-hydroxy-anthraquinone

[00301] Into a round bottom flask containing 50 mL of mesitylene was placed 5 mg of 2-allyl-1-hydroxy-anthraquinone. Nitrogen was bubbled for at least 30 minutes. Subsequently, a H-terminated silicon wafer was placed into the flask and was allowed to react with 2-allyl-1-hydroxy-anthraquinone for 12 hours under reflux at 150 °C in an oil bath. During the reaction, the solution was purged with nitrogen gas to prevent the substrate from being re-oxidized. Following the reaction, the 2-allyl-1-hydroxy-anthraquinone derivatized silicon wafer was rinsed with dichloromethane, hexane, then methanol and dried under a stream of nitrogen.

Example 12: Derivatization of silicon wafers with ferrocene using a semiconductor oxide surface

[00302] Si(100) wafer pieces were cleaned using "Piranha" solution (3:1 v/v concentrated H₂SO₄/30% H₂O₂) for 30 minutes at 100 °C and rinsed thoroughly with deionized water. Subsequently, the wafers were oxidized in 2:1:8 HCl/H₂O₂/H₂O for 15 minutes at 80 °C followed by 2:1:8 NH₄OH/H₂O₂/H₂O for 15 minutes at 80 °C and then rinsed with copious amounts of water. The oxidized silicon wafers were then immersed in 2% 3-aminopropyltriethoxysilane (APTES) solution in acetone for 2 minutes followed by rinsing with acetone to form an amino-terminated monolayer. This was then allowed to react with 10 mg/mL dicyclohexyl-carbodiimide (DCC) and 50 mg/mL ferrocene carboxylic acid in DMSO for 12 hours. Following the reaction, the ferrocene derivatized silicon wafer was rinsed with dichloromethane, acetone, and methanol and then dried under a stream of nitrogen.

Example 13: Derivatization of silicon wafers with anthracene using a semiconductor oxide surface

[00303] Si(100) wafer pieces were cleaned using "Piranha" solution (3:1 v/v concentrated H₂SO₄/30% H₂O₂) for 30 minutes at 100 °C and rinsed thoroughly with deionized water. Subsequently, the wafers were oxidized in 2:1:8 HCl/H₂O₂/H₂O for 15 minutes at 80 °C followed by 2:1:8 NH₄OH/H₂O₂/H₂O for 15 minutes at 80 °C and then rinsed with copious amounts of water. The oxidized silicon wafers were then immersed in 2% 3-APTES solution in acetone for 2 minutes followed by rinsing with acetone to form an amino-terminated monolayer. This was then allowed to react with 10 mg/mL dicyclohexyl-carbodiimide (DCC) and 50 mg/mL anthracene carboxylic acid in DMSO for 12 hours. Following the reaction, the anthracene derivatized silicon wafer was rinsed with dichloromethane, acetone and methanol and then dried under a stream of nitrogen.

Example 14: Ferrocene covalently attached to various doped Si(100) substrates

[00304] Ferrocene was covalently attached to the following four silicon surfaces via an H-terminated silicon substrate and vinyl ferrocene: i) Si (100) N-type (0.02-0.05 ohm cm) – highly-doped N-type, ii) Si (100) P-type (0.005-0.020 ohm cm) – highly-doped P-type, iii) Si (100) N-type (10-40 ohm cm) – lightly-doped N-type, and iv) Si (100) P-type (10-90 ohm cm) – highly-doped P-type. A well-defined ferrocene peak was observed for all four substrates. The highly-doped substrates in general produced a larger electrochemical current than the corresponding lightly-doped substrates. While not being bound by theory, this difference may be explained by the fact that the highly-doped substrates contain more charge carriers, i.e. are more conductive. Similar observations were observed when the electrochemistry was performed in a solution of ferrocene carboxaldehyde. Figure 19 has charts showing the peak current of silicon substrates derivatized with (a) vinyl ferrocene and (b) ferrocene carboxaldehyde in pH 1.63 solution on the four types of doped silicon.

Example 15: Four electrode system

[00305] A system was constructed having four electrodes comprising (i) a counter electrode, (ii) a reference electrode, (iii) a highly-doped N-type silicon wafer derivatized with vinyl ferrocene, and (iv) a

lightly-doped P-type silicon wafer derivatized with anthracene carboxaldehyde. Figure 20 illustrates the voltammograms obtained with the four electrode system consisting of a highly-doped N-type silicon wafer derivatized with vinyl ferrocene and a lightly-doped P-type silicon wafer derivatized with anthracene carboxaldehyde. The voltammograms were obtained in pH 7.03 solution and the peak potentials were stable over 200 consecutive scans (every 50th scan is shown).

[00306] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A sensor for detecting the presence of an analyte comprising:

5 a semiconductor electrode having a surface having immobilized thereon a redox-active moiety, wherein the redox-active moiety exhibits an oxidation potential and/or a reduction potential that is sensitive to the presence of said analyte.

2. The sensor of claim 1 wherein the analyte is hydrogen ion and the redox-active moiety is sensitive to hydrogen ion concentration.

10 3. The sensor of claim 1 wherein the sensor comprises a plurality of redox-active moieties, wherein at least one of the redox-active moieties is sensitive to the presence of an analyte, and at least one other redox-active moiety is substantially insensitive to the presence of said analyte.

4. The sensor of claim 3 wherein the analyte is hydrogen ion, and the moiety that is substantially insensitive to the presence of hydrogen ion has a substituent selected from the group consisting of
15 ferrocene, polyvinylferrocene, viologen, polyviologen, and polythiophene.

5. The sensor of claim 4 wherein the moiety that is substantially insensitive to the presence of hydrogen ion is ferrocene or a derivative of ferrocene.

6. The sensor of claim 1 wherein the analyte is hydrogen, and the redox-active moiety that is sensitive to the presence of the hydrogen ion comprises a substituent selected from the group
20 consisting of anthracenes, quinones, anthroquinones, phenanthroquinones, phenylene diamines, catechols, phenothiazinium dyes, monoquaternized N-alkyl-4,4'-bipyridinium, RuO_x , and $\text{Ni}(\text{OH})_2$.

7. The sensor of claim 6 wherein the redox-active moiety that is sensitive to the presence of hydrogen ion comprises a substituent comprising anthracene.

8. The sensor of claims 6 wherein the redox-active moiety that is sensitive to the presence of hydrogen ion comprises a substituent comprising an anthraquinone or a phenanthraquinone.

9. The sensor of claim 1 wherein the redox-active moiety that is sensitive to the presence of an analyte is sensitive to the concentration of the analyte.

10. The sensor of claim 9 wherein the oxidation potential and/or reduction potential of the redox-active moiety is sensitive to the concentration of the analyte in a range from 10^{-3} M to 10^{-10} M.

11. The sensor of claim 10 wherein the oxidation potential and/or reduction potential of the redox-active moiety is sensitive to the concentration of the analyte in a range from 10^{-1} M to 10^{-14} M.

12. The sensor of claim 11 wherein the sensor detects hydrogen ion concentration from pH3 to pH10.

13. The sensor of claim 11 wherein the sensor detects hydrogen ion concentration from pH1 to pH14.

14. The sensor of claim 11 wherein the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.1 pH unit.
15. The sensor of claim 11 wherein the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.01 pH units.
- 5 16. The sensor of claim 1 wherein the redox-active moiety is covalently bound to the surface of the electrode.
17. The sensor of claim 1 wherein the redox-active moiety is bound to a polymer that is immobilized onto the surface of the electrode.
18. The sensor of claim 1 wherein the semiconductor electrode is doped.
- 10 19. The sensor of claim 18 wherein the semiconductor electrode is P-doped.
20. The sensor of claim 19, wherein the semiconductor electrode comprises a silicon electrode doped with boron.
21. The sensor of claim 18 wherein the semiconductor electrode is N-doped.
22. The sensor of claim 21, wherein the semiconductor electrode comprises a silicon electrode is
15 doped with phosphorous.
23. The sensor of claim 1 wherein the semiconductor electrode comprises a monolithic piece of silicon.
24. The sensor of claim 1 wherein the semiconductor electrode comprises a composite electrode, said composite electrode comprising semiconductor particles in a matrix.
- 20 25. The sensor of claim 24, wherein the semiconductor electrode comprises a composite electrode bound to a conductive substrate.
26. The sensor of claim 25 wherein the semiconductor particles are present in an amount such that a conductive path is created across the composite electrode.
27. The sensor of claim 1 wherein the electrode comprises silicon.
- 25 28. The sensor of claim 1 wherein the electrode comprises unpolished silicon.
29. The sensor of claim 1 wherein the electrode comprises polished silicon.
30. The sensor of claim 1 wherein the semiconductor electrode has a resistivity within the range of 0.01 to 1000 Ω -cm.
31. The sensor of claim 1 wherein the semiconductor electrode has a resistivity within the range of 1
30 to 100 Ω -cm.
32. The sensor of claim 3 wherein the sensor is capable of measuring analyte concentration without calibration with an external standard.

33. The sensor of claim 32 wherein the sensor remains sensitive to the analyte without calibration after a first use by an end user.
34. The sensor of claim 32 wherein the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 6 days.
- 5 35. The sensor of claim 34 wherein the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after exposure to the cell culture medium for at least 6 days.
36. The sensor of claim 1 wherein the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 40 minutes.
- 10 37. The sensor of claim 36 wherein the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 400 minutes.
38. The sensor of claim 1 wherein the semiconductor substrate has a plurality of zones wherein at least a first zone is sensitive to an analyte, and a second zone that is insensitive to an analyte.
39. An analyte-sensing system comprising:
- 15 (a) a working electrode having a semiconductor surface that has immobilized thereon a redox-active moiety, wherein the redox-active moiety has an oxidation potential and/or reduction potential that is sensitive to the presence of said analyte;
- (b) a counter electrode and optionally a reference electrode;
- (c) a source for supplying a plurality of potentials to the working electrode; and
- (d) a device for measuring current through the working electrode at the plurality of potentials.
- 20 40. The system of claim 39, further comprising a second working electrode comprising a second semiconductor substrate comprising a second redox-active moiety having an oxidation potential and/or reduction potential that is insensitive to the presence of said analyte.
41. The system of claim 39 wherein the source for supplying a plurality of potentials is a potentiostat capable of applying square waves for square wave voltammetry.
- 25 42. The system of claim 41 further comprising a computation system that communicates with the device for measuring current, and that calculates a reduction or oxidation potential from the measured current at a plurality of potentials.
43. The system of claim 42 wherein the system is used as an in-line sensor in a process.
44. The system of claim 43 wherein the currents measured at a plurality of potentials are used to determine analyte concentration, and the determined analyte concentration is used to control a process parameter.
- 30 45. The system of claim 39 wherein the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.1 pH unit.

46. The system of claim 39 wherein the sensor measures hydrogen ion concentration within an accuracy of plus or minus 0.01 pH units.
47. The system of claim 40 wherein the sensor is capable of measuring analyte concentration without calibration with an external standard.
- 5 48. The system of claim 40 wherein the sensor remains sensitive to the analyte without calibration after a first use by an end user.
49. The system of claim 40 wherein the analyte is hydrogen ion and the sensor remains sensitive to hydrogen ion after exposure to a cell culture medium for at least 6 days.
- 10 50. The system of claim 49 wherein the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after exposure to the cell culture medium for at least 6 days.
51. The system of claim 40 wherein the analyte is hydrogen ion and the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 40 minutes.
- 15 52. The system of claim 51 wherein the sensor is capable of measuring pH with an accuracy of plus or minus 0.2 units after autoclave treatment at 121°C for 400 minutes.
53. A semiconductor substrate having a surface, wherein the surface comprises a redox-active moiety immobilized thereon, said redox-active moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of an analyte.
- 20 54. The semiconductor substrate of claim 53, wherein immobilized thereon is also a second redox-active moiety having an oxidation potential and/or a reduction potential that is substantially insensitive to the presence of the analyte.
55. The semiconductor substrate of claim 53, wherein the analyte is an ion.
56. The semiconductor substrate of claim 53, wherein the analyte is hydrogen ion.
- 25 57. The semiconductor substrate of claim 53 wherein the semiconductor comprises an inorganic semiconductor.
58. The semiconductor substrate of claim 53 wherein the semiconductor comprises an organic semiconductor.
59. The semiconductor substrate of claim 57 wherein the inorganic semiconductor comprises silicon or gallium arsenide.
- 30 60. The semiconductor substrate of claim 58 wherein the organic semiconductor comprises polyacetylene, polythiophene, or polypyrrole.
61. The semiconductor substrate of claim 53 wherein the semiconductor comprises silicon.

62. The semiconductor substrate of claim 53 wherein the semiconductor comprises unpolished silicon.
63. The semiconductor substrate of claim 53 wherein the semiconductor comprises polished silicon.
64. The semiconductor substrate of claim 53 wherein the redox-active moiety is directly bound to the surface.
65. The semiconductor substrate of claim 64, wherein the redox-active moiety is covalently bound to the surface.
66. The semiconductor substrate of claim 64, wherein the redox-active moiety is covalently bound to the surface through a linker.
67. The semiconductor substrate of claim 53, wherein the redox-active moiety is covalently bound to a polymer that is immobilized onto the surface of the semiconductor substrate.
68. The semiconductor substrate of claim 67, wherein the redox-active moiety is covalently bound to a polymer that is covalently bound to the surface of the semiconductor substrate
69. The semiconductor substrate of claim 53 wherein the semiconductor is doped.
70. The semiconductor substrate of claim 69 wherein the semiconductor is N-doped.
71. The semiconductor substrate of claim 69 wherein the semiconductor is P-doped.
72. The semiconductor substrate of claim 53 wherein substrate comprises crystalline silicon wherein the surface displaying predominantly one crystalline plane.
73. The semiconductor substrate of claim 53 wherein the substrate has a plurality of zones wherein each zone comprises a redox-active moiety immobilized thereon, said redox-active moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of an analyte.
74. The semiconductor substrate of claim 73 wherein a first zone comprises a redox moiety sensitive to a first analyte, and a second zone comprises a redox moiety sensitive to a second analyte.
75. The semiconductor substrate of claim 54 further comprising a third redox-active moiety exhibiting an oxidation potential and/or a reduction potential that is sensitive to the presence of a second analyte.
76. The semiconductor substrate of claim 75 wherein the second analyte is ammonia, oxygen or carbon dioxide.
77. A method for forming an analyte-sensitive semiconductor electrode, said electrode having a surface, the method comprising:
immobilizing a redox-active moiety that is sensitive to the presence of an analyte onto the surface.

78. The method of claim 77 wherein immobilizing the redox-active moiety covalently binds the redox-active moiety to the surface.
79. The method of claim 78 wherein the redox-active moiety is covalently bound to the surface through a linker.
- 5 80. The method of claim 61, wherein immobilizing the redox-active moiety comprises hydrosilation, a free radical reaction, carbodiimide coupling, a Diels-Alder reaction, a Michael addition, or click chemistry.
81. The method of claim 77 wherein the redox-active moiety is covalently bound to a polymer that is immobilized onto the surface.
- 10 82. The method of claim 77 wherein the step of immobilizing comprises polymerization including a monomer or oligomer comprising a redox-active moiety.
83. The method of claim 82 wherein the polymerization of a monomer or oligomer comprising a redox-active moiety includes a reaction with a functional group covalently bound to the surface, whereby the polymer formed by polymerization is covalently bound to the surface.
- 15 84. The method of claim 82 wherein the monomer or oligomer is electropolymerized onto the surface.
85. The method of claim 81, wherein the step of immobilizing comprises coating or casting the polymer onto the surface.
86. The method of claim 77 wherein the semiconductor surface comprises a composite electrode that
20 comprises semiconductor particles within a matrix.
87. A method of forming a semiconductor surface derivatized with one or more redox-active moieties comprising:
contacting an H-terminated semiconductor surface with the one or more redox-active moieties
wherein at least one redox active moiety is sensitive to the presence of an analyte, and wherein
25 each redox-active moiety comprises a functional group that will react with the H-terminated semiconductor surface to form a covalently bond, thereby forming a derivatized semiconductor surface.
88. The method of claim 87 wherein the semiconductor surface comprises silicon.
89. The method of claim 87 wherein at least two redox active moieties are used, and one of the redox
30 active moieties is insensitive to the presence of the analyte.
90. The method of claim 87 wherein the H-terminated semiconductor surface is formed by treatment with hydrofluoric acid.
91. The method of claim 87 wherein the functional group is a vinyl group.

92. The method of claim 87 wherein the functional group is an aldehyde group.

93. The method of claim 87 wherein the functional group is a diazonium group.

94. A method of determining the concentration of an analyte, comprising

(a) placing an electrode in contact with said analyte, said electrode comprising a semiconductor substrate with a semiconductor surface having immobilized thereon an analyte-sensitive redox-active moiety, said analyte-sensitive redox-active moiety exhibiting an oxidation potential and/or reduction potential that is sensitive to the concentration of the analyte;

(b) applying a plurality of potentials to the electrode; and

(c) measuring the current through the electrode at the plurality of potentials to determine a reduction and/or oxidation potential of the analyte-sensitive redox-active moiety, thereby determining the concentration of the analyte.

95. The method of claim 94, wherein the measuring of the current at the plurality of potentials provides a peak current, and whereby the peak current is used to determine reduction and/or oxidation potential of the analyte-sensitive redox-active moiety.

96. The method of claim 94, wherein the analyte is hydrogen ion.

97. The method of claim 94, further comprising an analyte-insensitive redox-active moiety bound to an electrode comprising a semiconductor substrate, such redox-active moiety having a reduction and/or oxidation potential that is substantially insensitive to the analyte, further comprising determining the oxidation and/or reduction potential of the analyte-insensitive redox-active moiety, and determining the concentration of the analyte from the difference in the oxidation and/or reduction potentials of the analyte-sensitive and analyte-insensitive moieties.

98. The method of claim 94 wherein the analyte is provided in a solution.

99. A method comprising:

(a) measuring a pH value of a step in a water or waste treatment process with a voltammetric pH sensor, wherein the pH sensor comprises a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion; and

(b) using the pH value to monitor or control the treatment process.

100. A method comprising:

(a) measuring a pH value of a reaction mixture in a biopharmaceutical process with a voltammetric pH sensor, wherein the pH sensor comprises a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration to obtain a pH value; and

(b) using the pH value to monitor the biopharmaceutical process.

101. The method of claim 100 wherein the pH value is measured on a sample obtained from the reaction mixture.

102. A reactor for carrying out a biopharmaceutical process wherein the reactor comprises a pH sensor having a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration.

103. The reactor of claim 102 wherein the pH sensor is a voltammetric pH sensor.

104. The reactor of claim 102 wherein the reactor is a disposable bioreactor.

105. The reactor of claim 104 wherein the reactor is a bioprocess flexible container.

106. A method for carrying out an industrial process comprising:

(a) measuring a pH value of a step of an industrial process with a voltammetric pH sensor having a redox-active moiety that is sensitive to hydrogen ion concentration, and a redox-active moiety that is substantially insensitive to hydrogen ion concentration; and

(b) using the pH value to carry out the industrial process.

107. A sensor for measuring ion concentration in a bodily fluid within a body comprising:

an electrode configured to be in contact with a bodily fluid, said electrode comprising a semiconductor surface that has immobilized thereon a redox-active moiety, wherein the redox-active moiety has an oxidation potential and/or reduction potential that is sensitive to concentration of said ion.

108. A method of measuring concentration in a bodily fluid within a body comprising:

placing the sensor of claim 107 in contact with the bodily fluid; and

operating the sensor to yield a value of the concentration of the ion present in said bodily fluid.

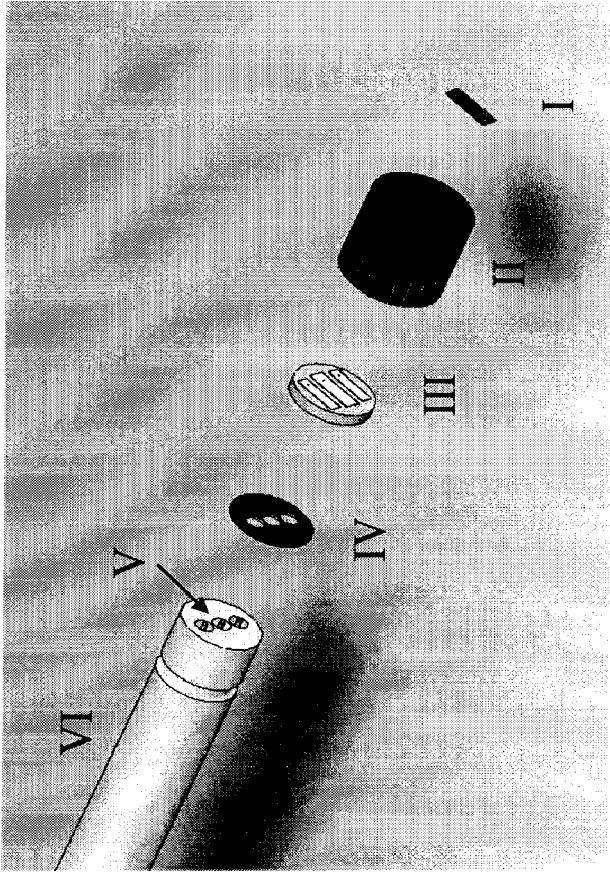
109. A bioreactor comprising a reservoir for containing a reaction mixture and a pH probe wherein the pH probe comprises an electrode having a semiconductor surface, the semiconductor surface having immobilized thereon a redox active moiety having a reduction and/or oxidation potential that is sensitive to the presence of hydrogen ion.

110. The bioreactor of claim 109 wherein the probe further comprises a semiconductor surface having immobilized thereon a redox active moiety having a reduction and/or oxidation potential that is insensitive to the presence of hydrogen ion.

111. The bioreactor of claim 109 wherein the semiconductor surface on which the redox active moiety having a reduction and/or oxidation potential that is sensitive to the presence of hydrogen ion is immobilized is the same semiconductor surface on which the redox active moiety having a reduction and/or oxidation potential that is insensitive to the presence of hydrogen ion is immobilized on.

Figure 1

(a)



(b)

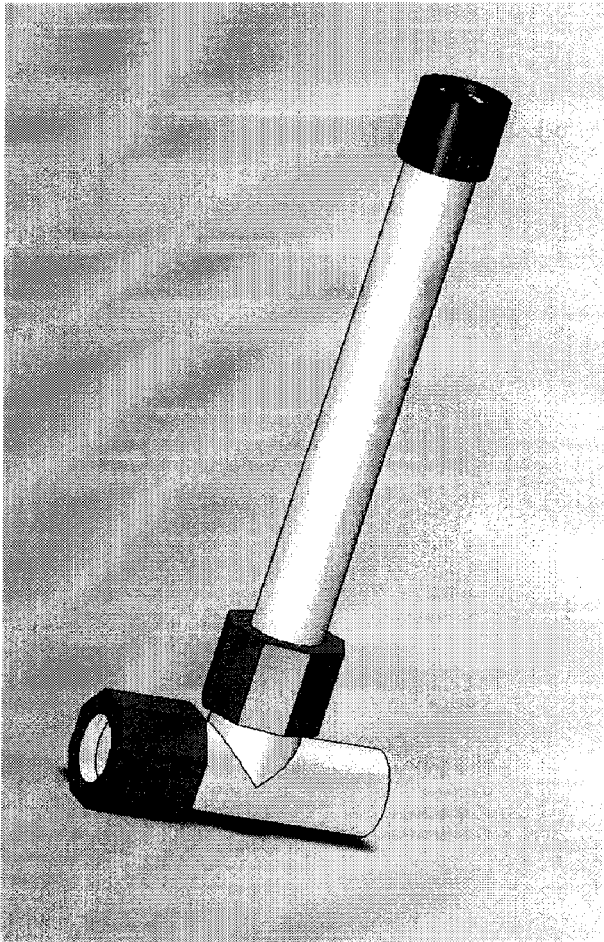


Figure 2

(a)



(b)

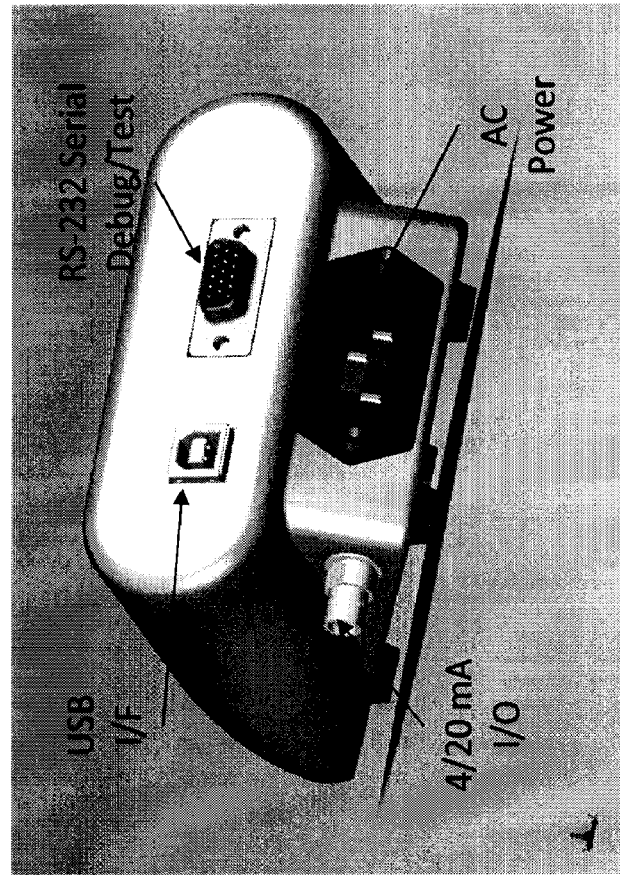


Figure 3

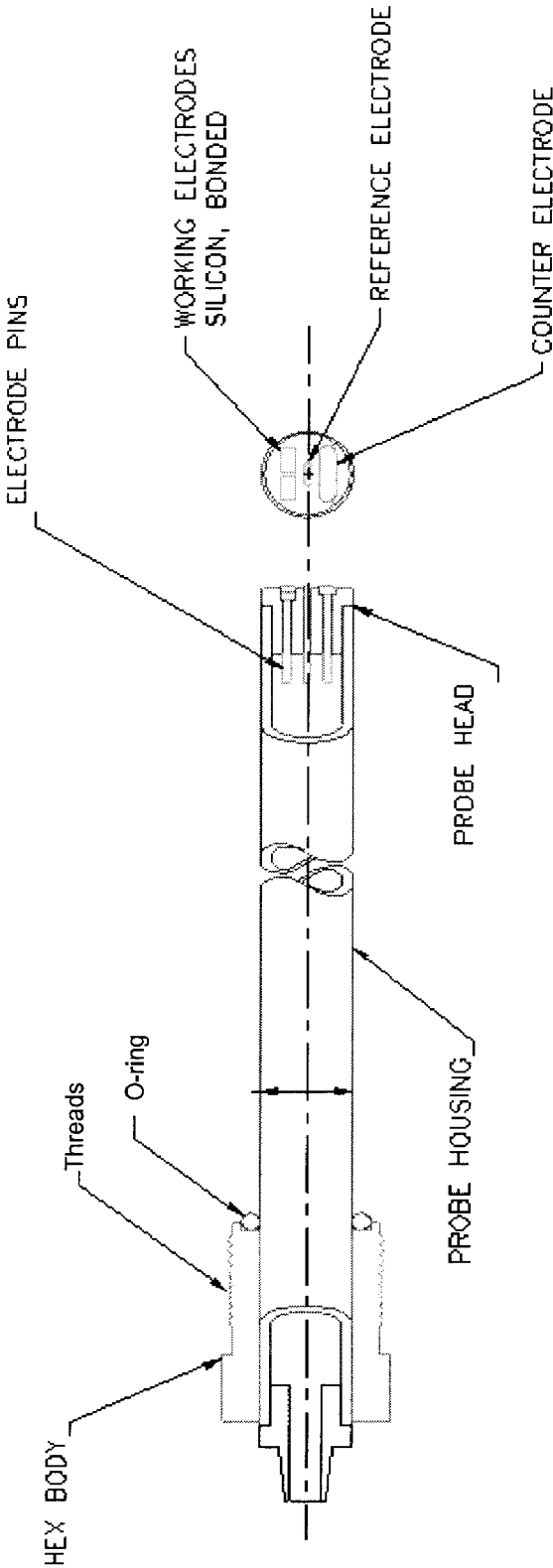


Figure 4

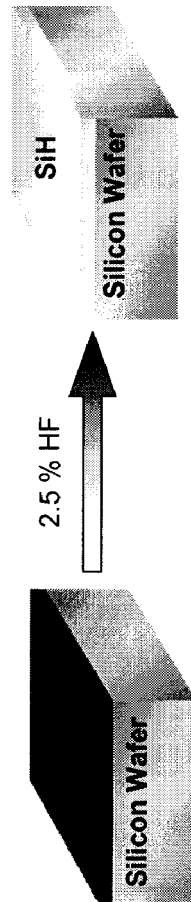


Figure 5

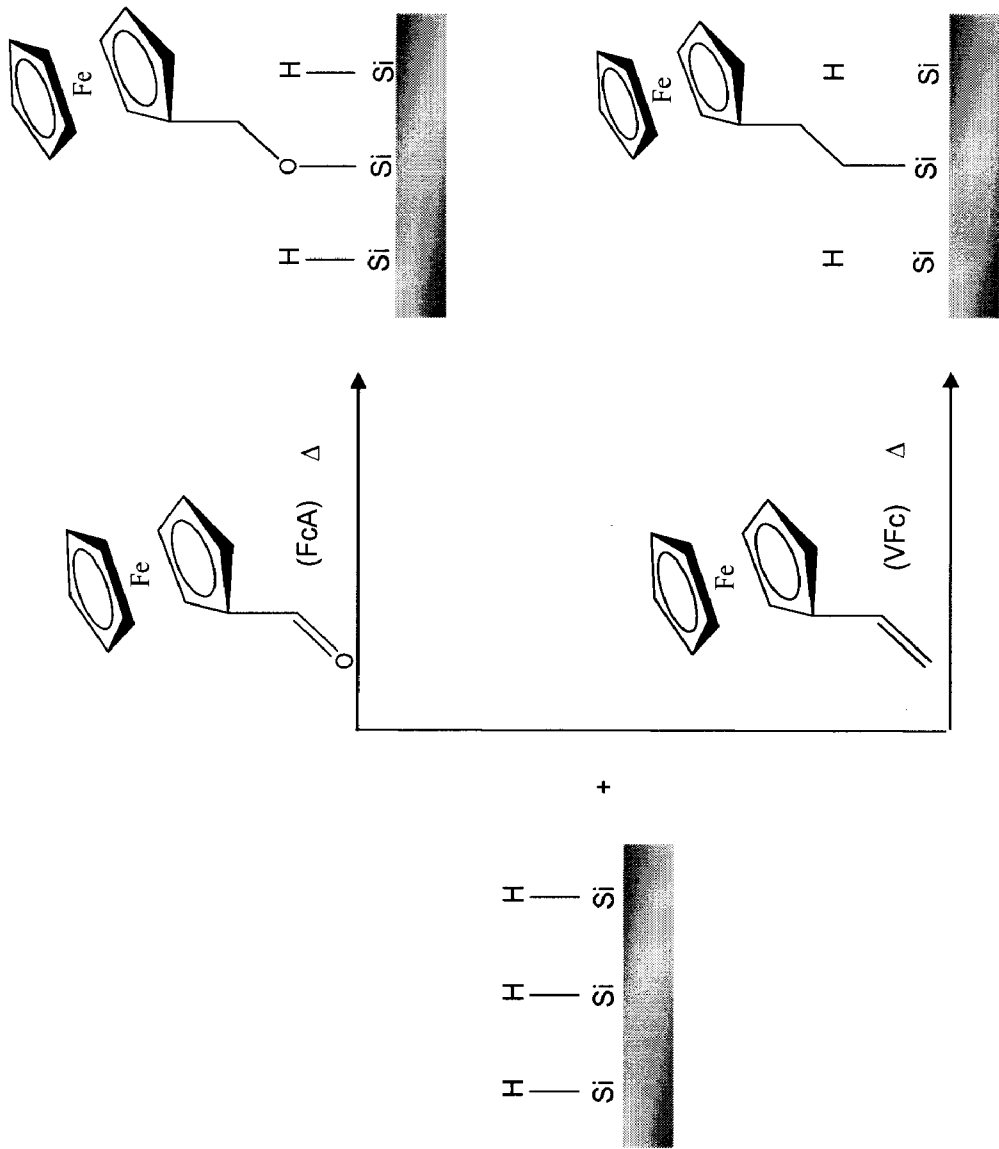


Figure 6

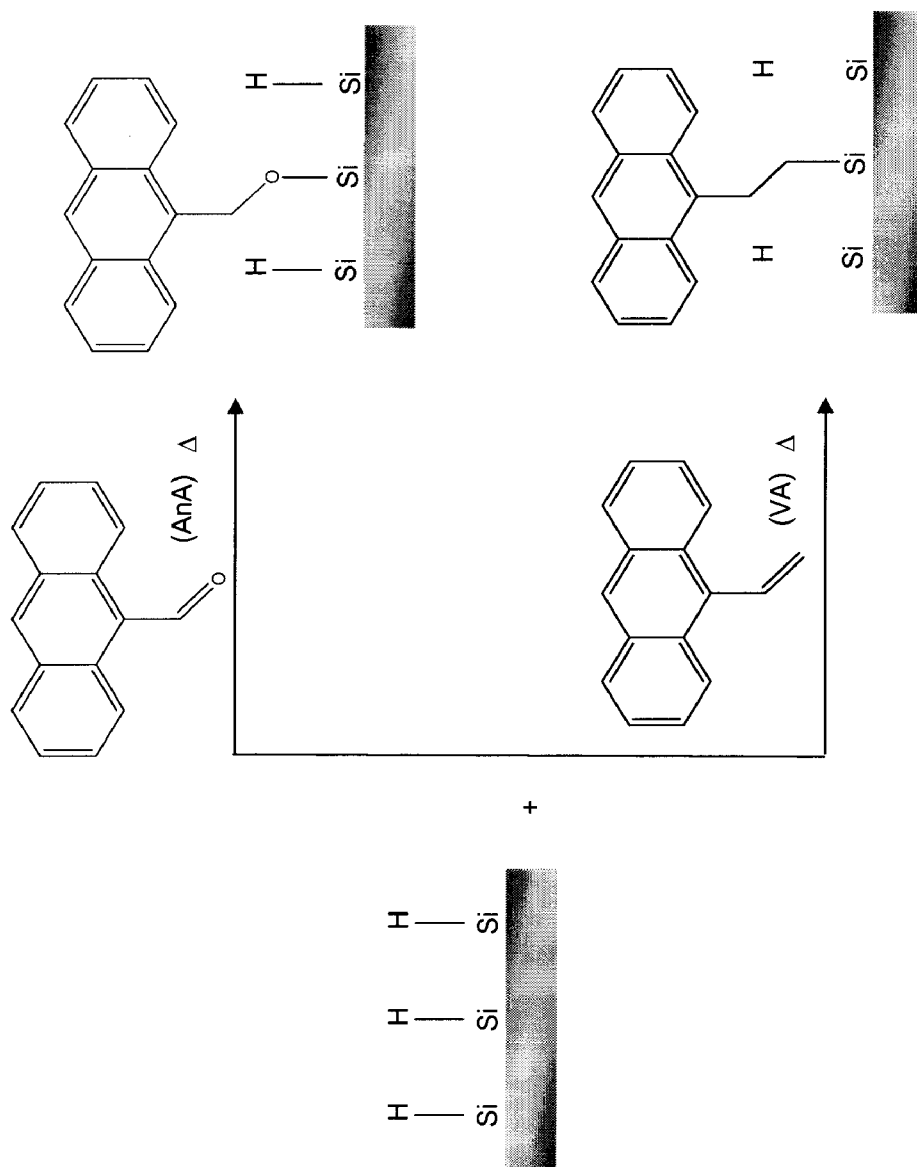


Figure 7

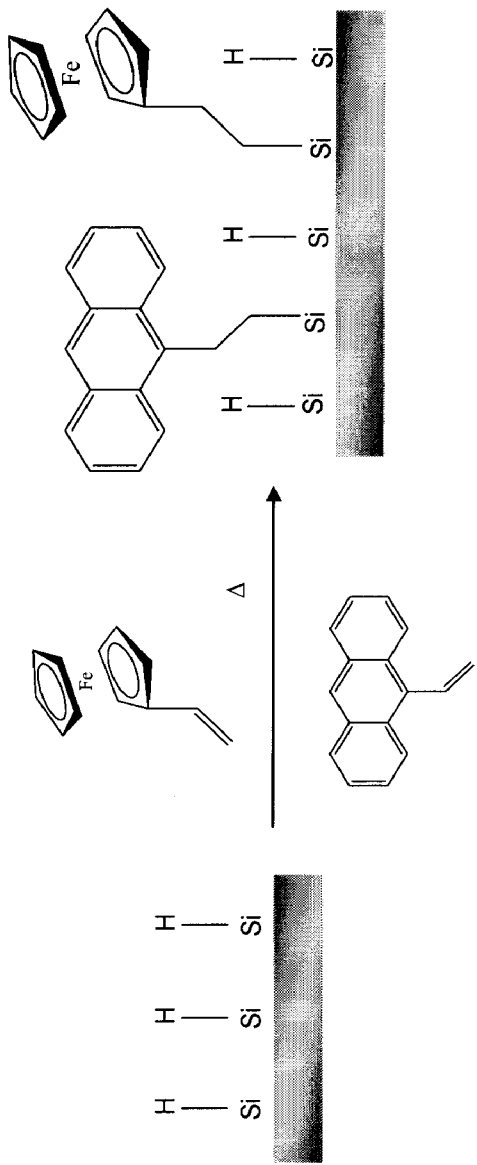


Figure 8

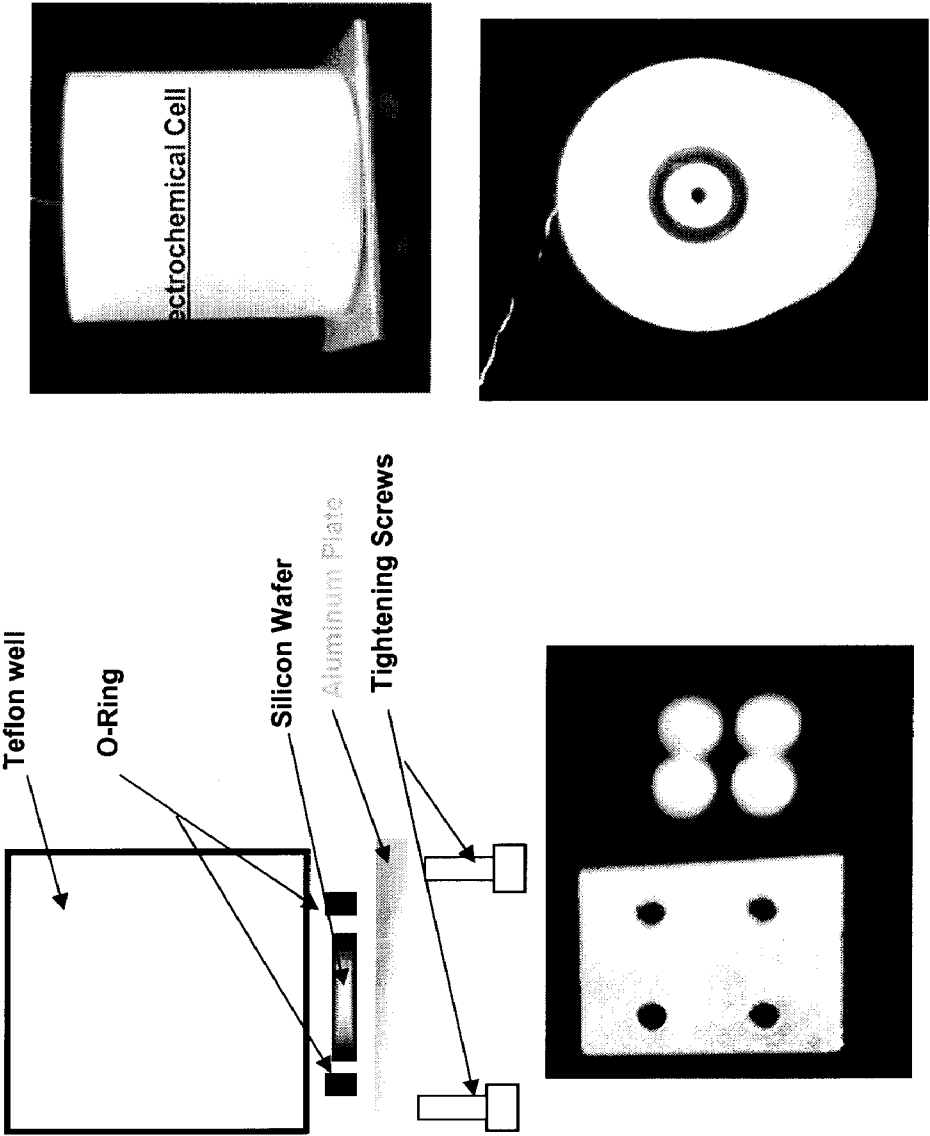


Figure 9

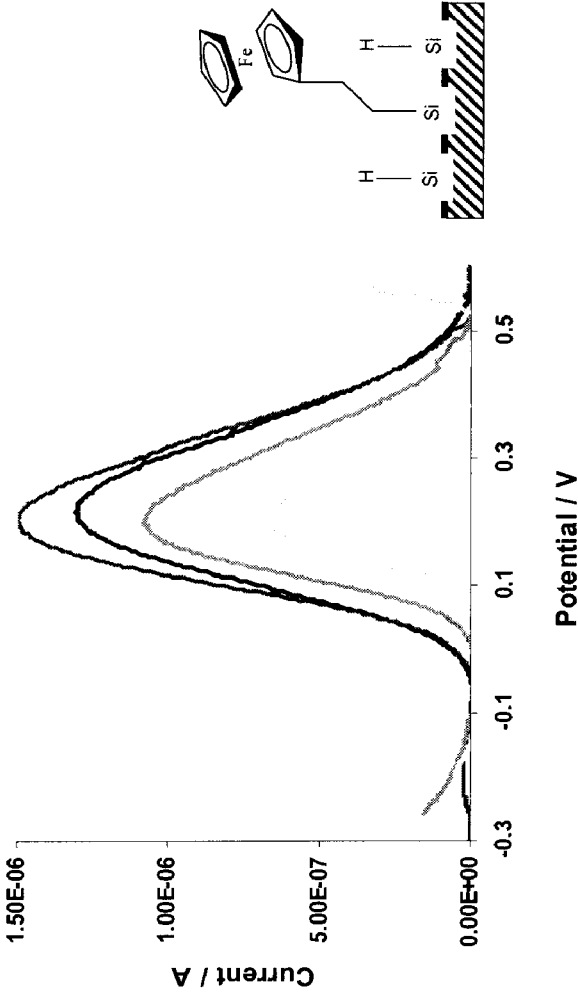


Figure 10

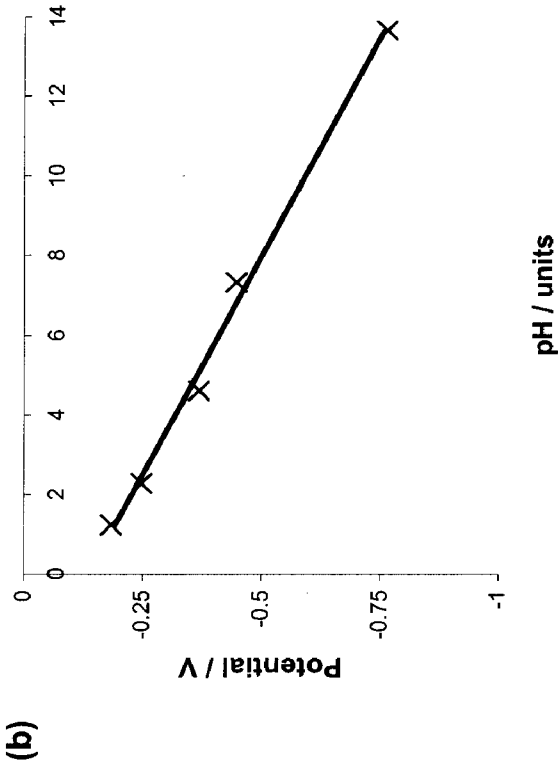
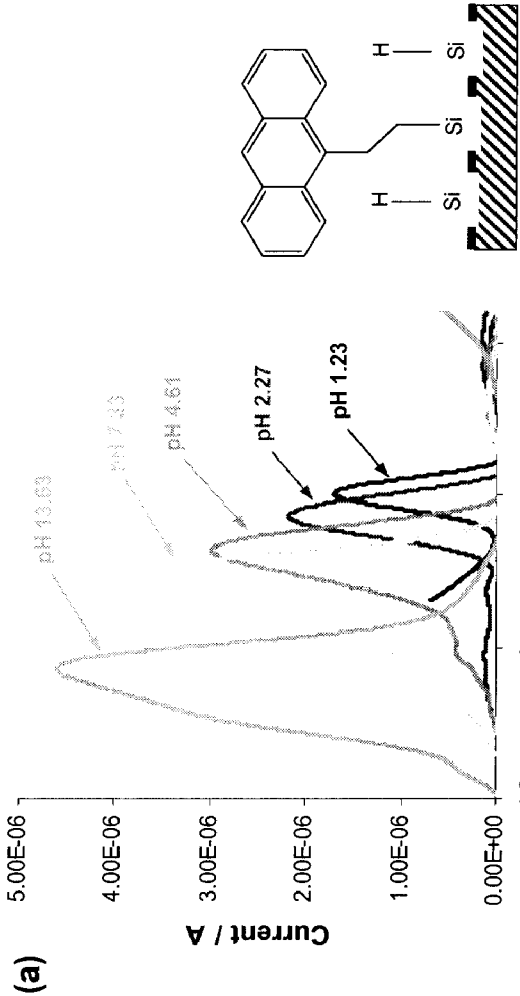


Figure 11

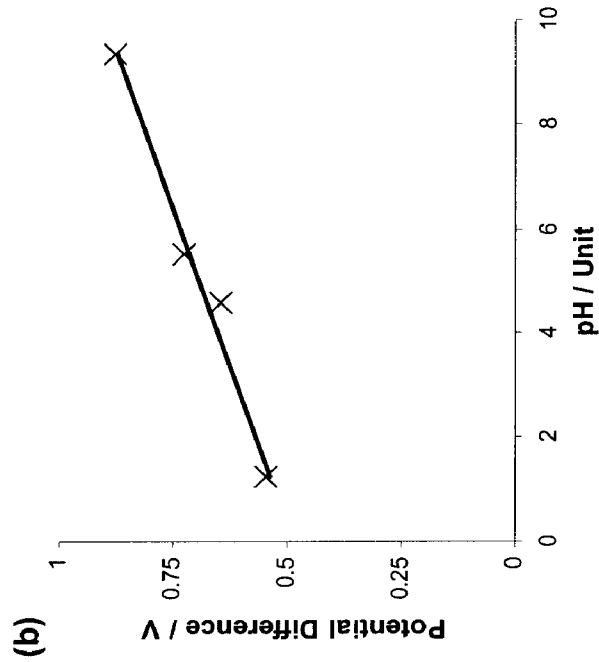
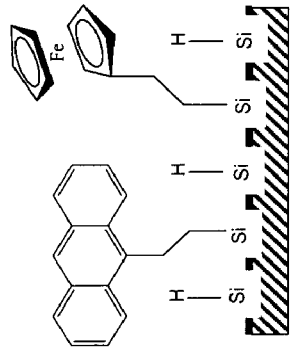
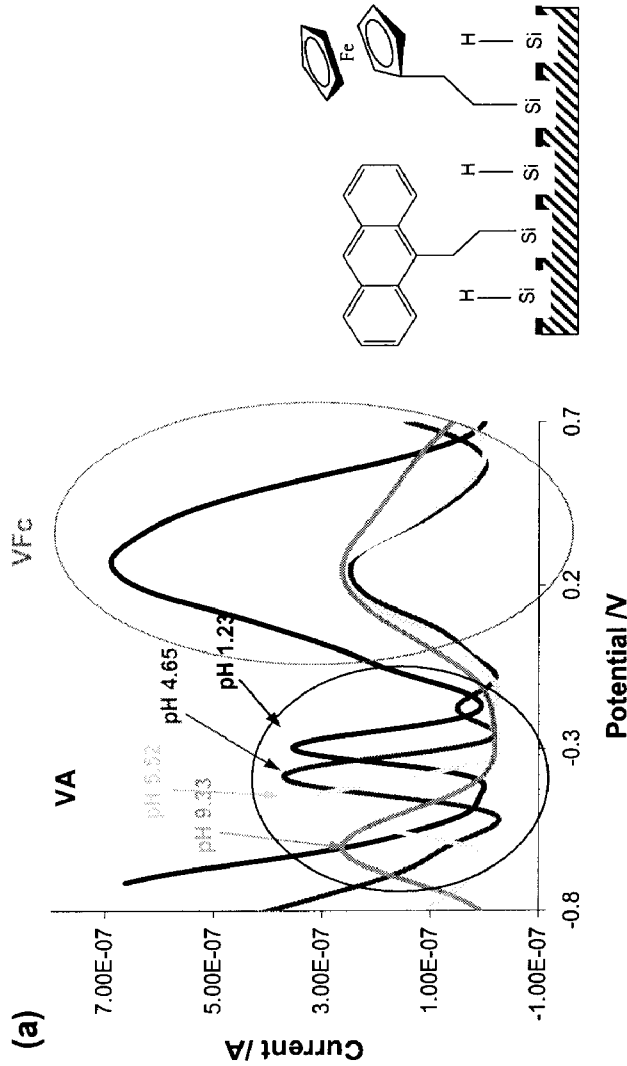


Figure 12

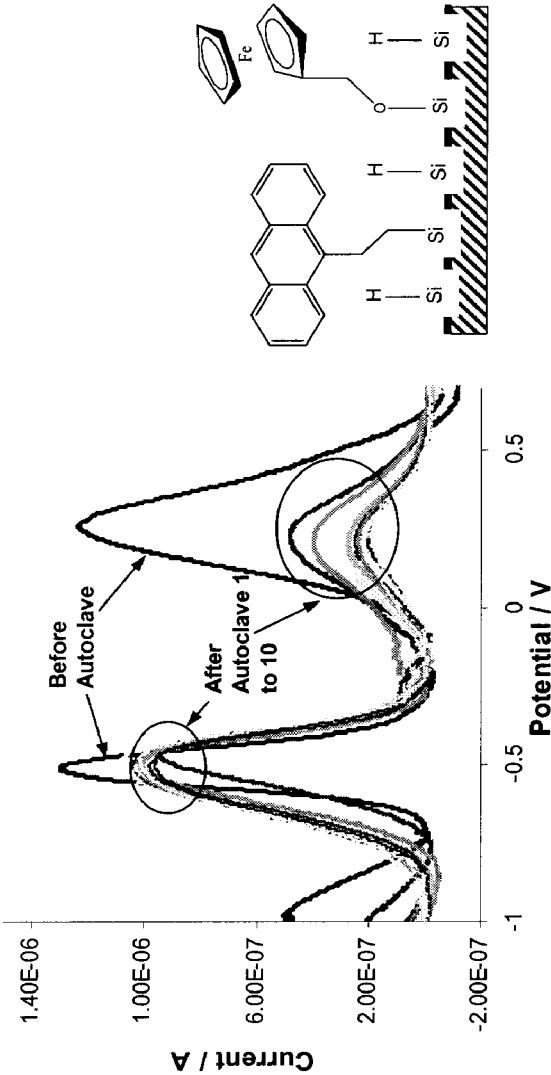


Figure 13

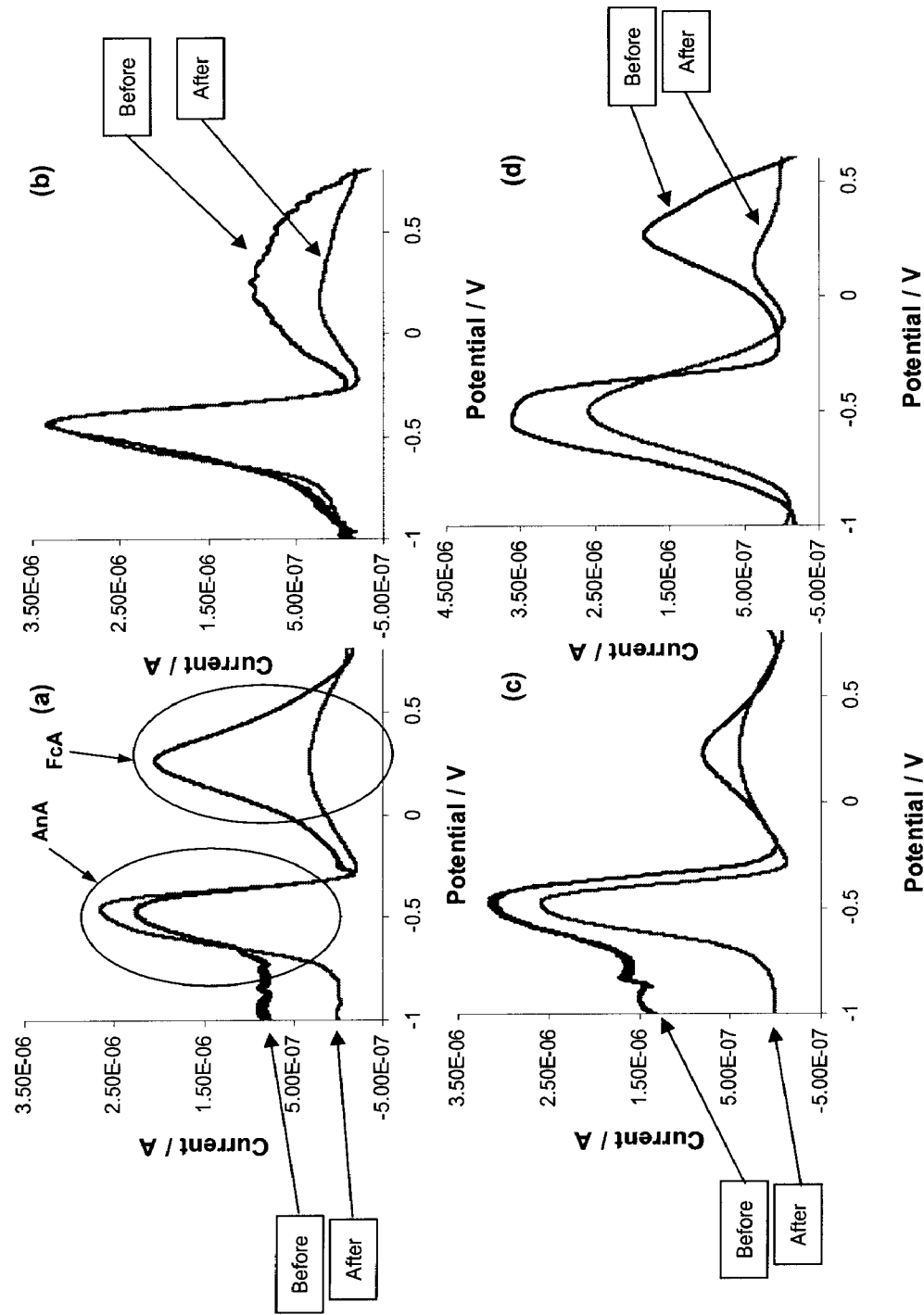


Figure 14

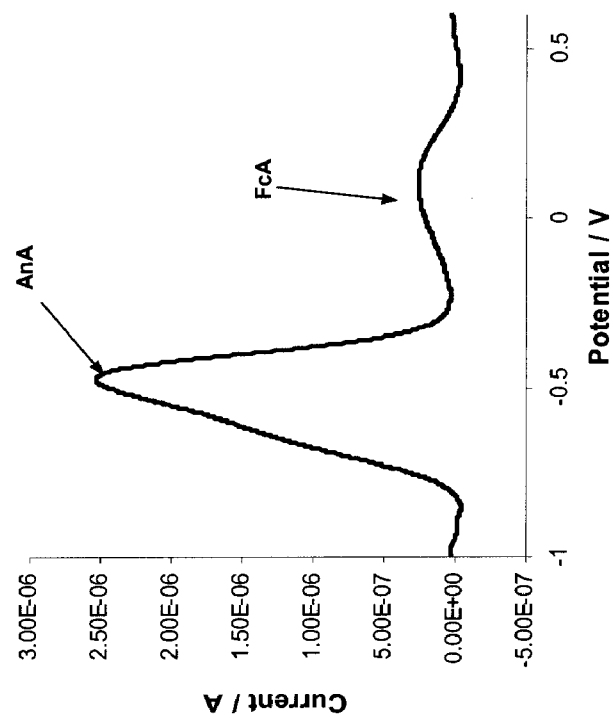
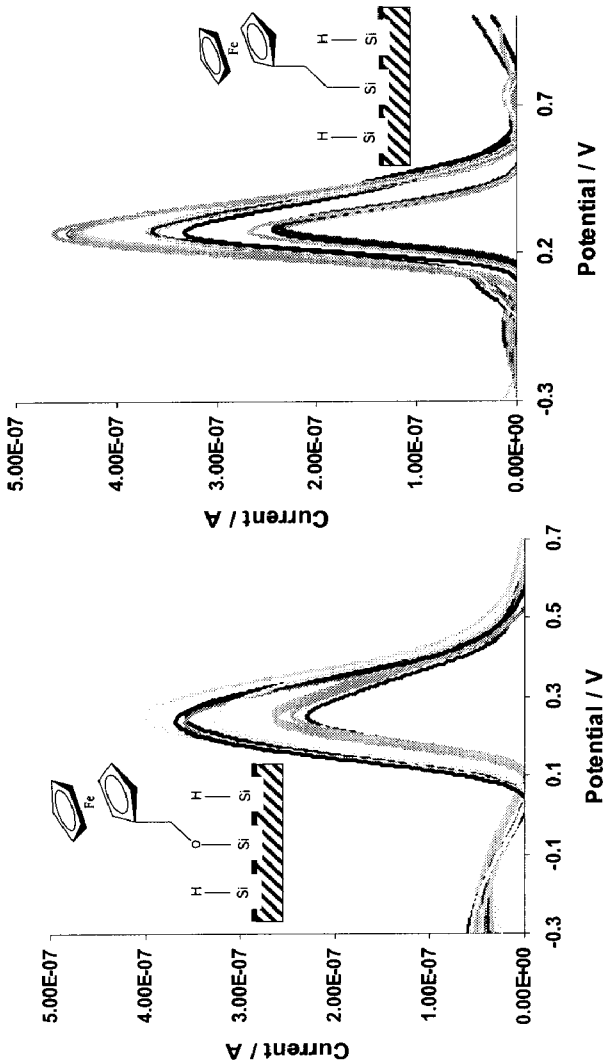


Figure 15



(b)

(a)

Figure 16

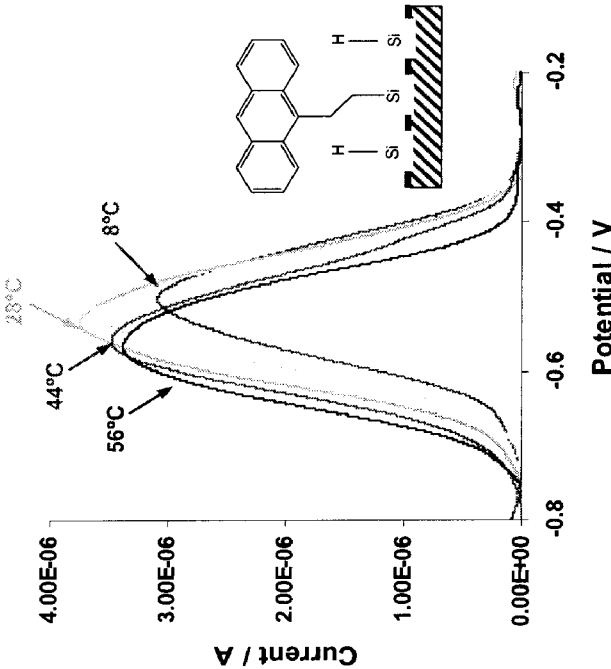


Figure 17

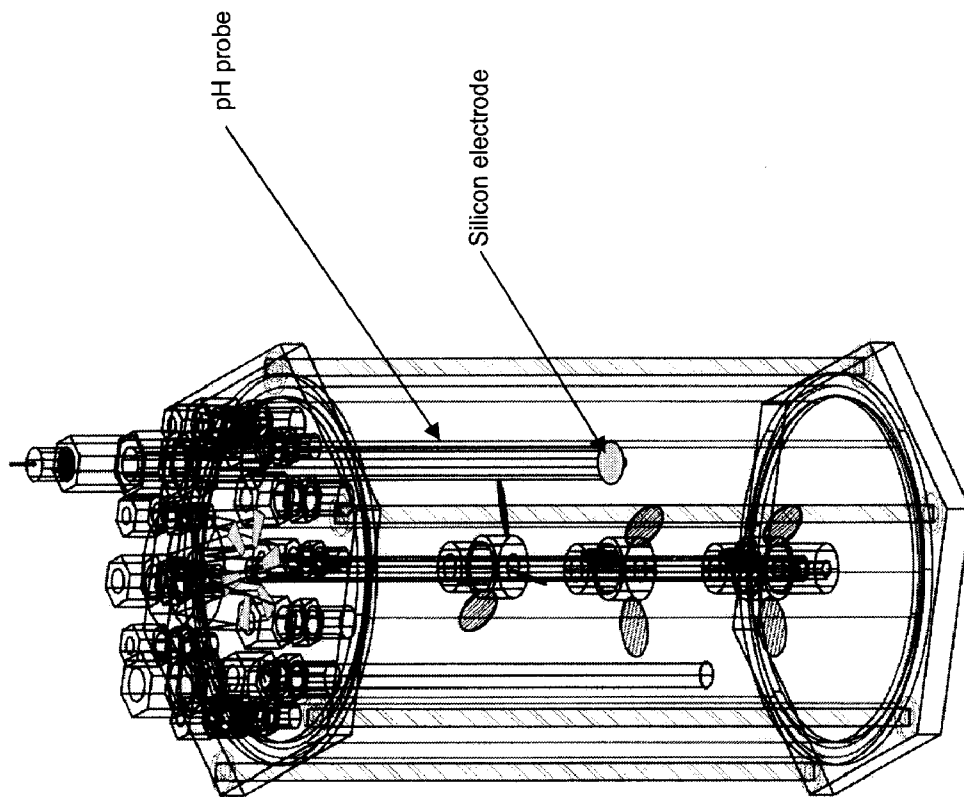
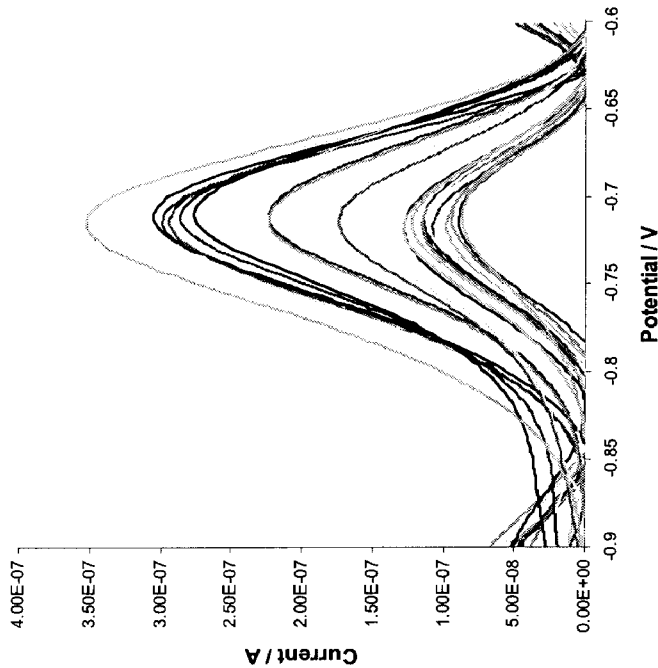
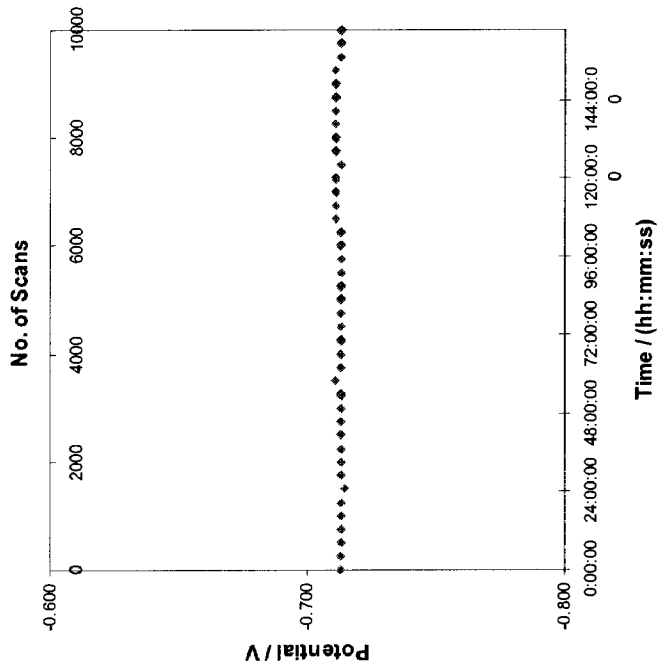


Figure 18



(a)



(b)

Figure 19

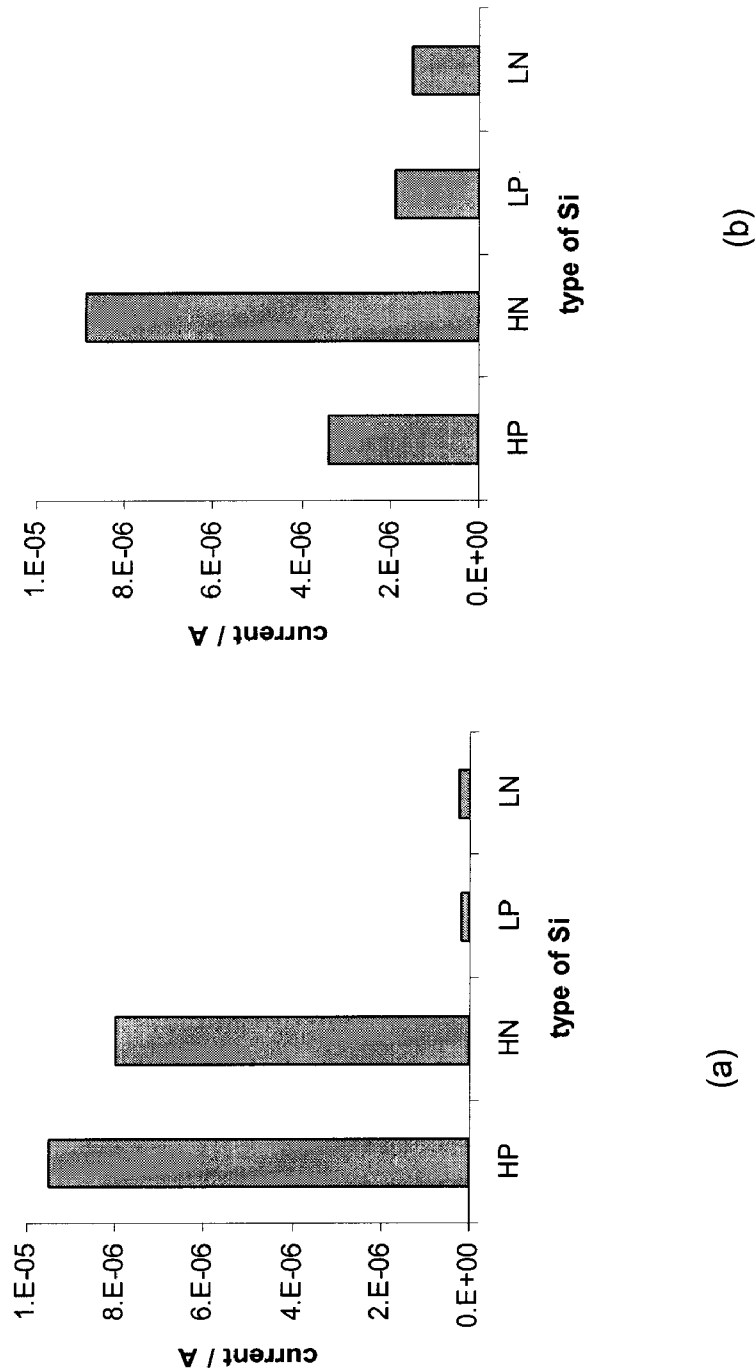


Figure 20

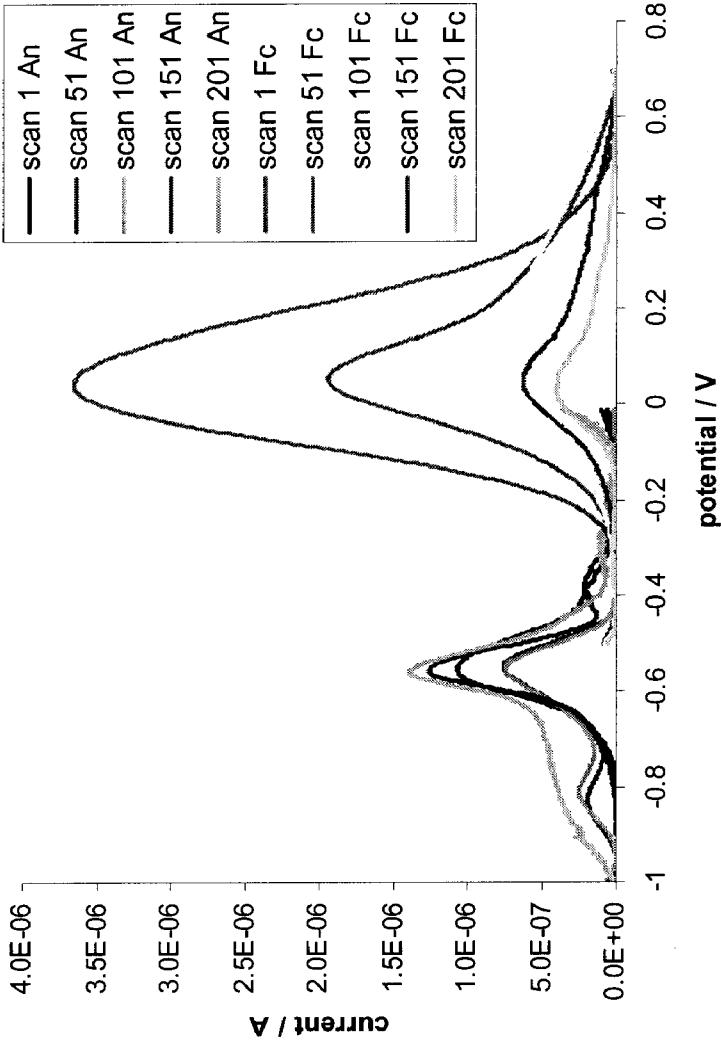
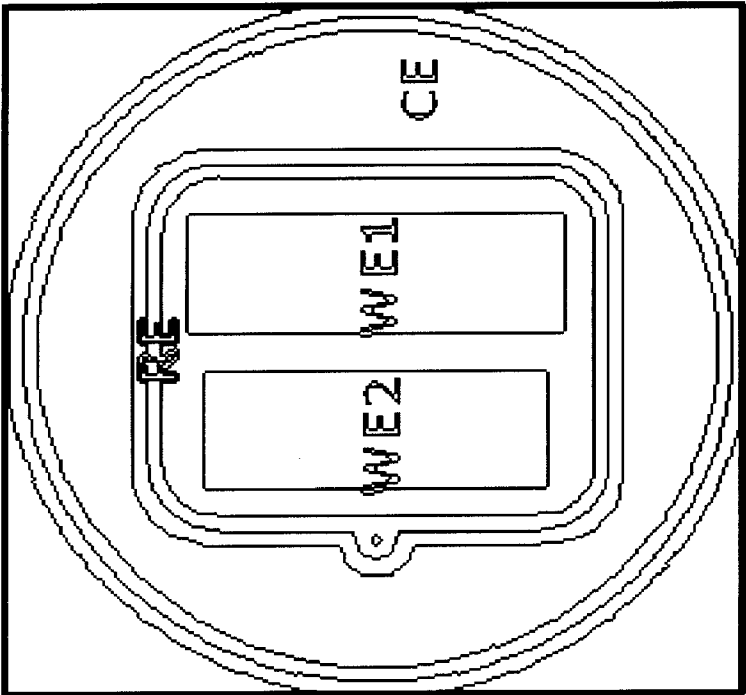
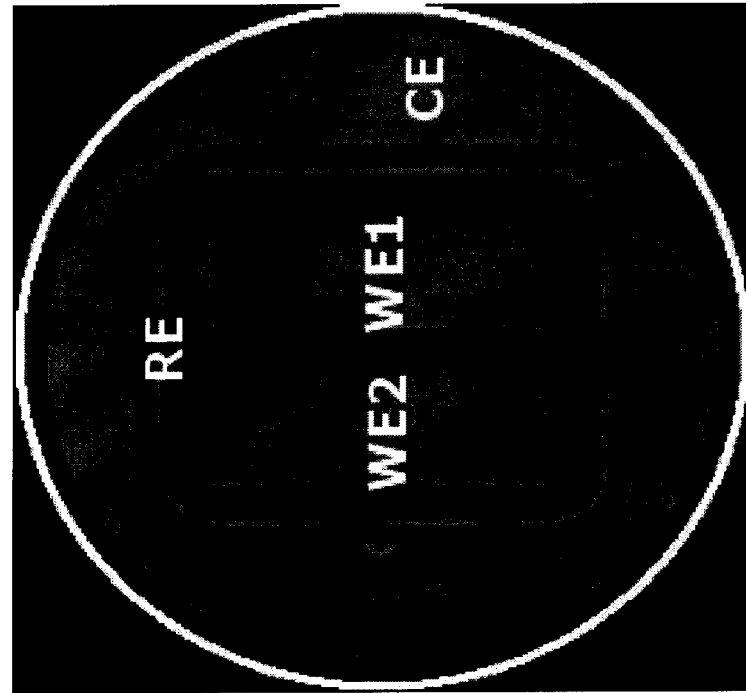


Figure 21



(b)



(a)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 08/66165

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 27/403 (2008.04)

USPC - 204/403.01, 204/406

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(8) - G01N 27/403 (2008.04)

USPC - 204/403.01, 204/406

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)

PubWEST, Google Patents Database, Google Scholar Database

(analyte, semiconductor, electrode, surface, immobilize, redox, active, moiety, oxidation, potential, hydrogen, ferrocene, polyvinyl, viologen, thiophene, derivative, anthracene, quinine, phenylene, diamine, catechol, phenothiazinium, ruthenium, pH, dope, boron)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,567,302 A (Song, et al.) 22 Oct 1996 (22.10.1996) column 1, line 18-22, line 24-27, column 5, line 63-67, column 6, line 16-19, line 37-42, line 50-55, line 65-66, column 7, line 4-6, line 9-13, line 16-20, column 8, line 17-23, line 54-67, column 9, line 8-10, line 56-59, column 10, line 5-13, column 12, line 66-67, column 13, line 1-3, line 9-12, column 15, line 4-5, line 61-65, column 16, line 15-17, line 52-55, column 17, line 18-20, line 23-33, column 18, line 38-40, column 19, line 13-17, column 22, line 15-20, column 24, line 3-6, line 63-65, column 26, line 67, column 27, line 1-5, line 16-18, line 35-37, column 41, line 64-66	1-3, 9-11, 17, 27-29, 38-40, 53-59, 61-64, 72-77, 94-98, 107-108
Y		4-8, 12-16, 18-26, 30-37, 41-52, 60, 65-71, 78-93, 99-106, 109-112
Y	US 6,340,588 B1 (Nova, et al.) 22 Jan 2002 (22.01.2002) column 9, line 5-8, line 54-58, column 10, line 7-13, column 11, line 33-34, column 12, line 24-26, column 13, line 30-35, column 14, line 31-35, column 21, line 16-19, column 24, line 43-44, column 31, line 66-67, column 32, line 1, line 27-32, column 36, line 30-33, column 39, line 58-62, line 65-67, column 40, line 1-3, column 45, line 49-57, column 54, line 7-11, column 55, line 52-54, column 57, line 55-57, column 58, line 1-8, line 11-16, line 42-46, line 62-63, column 88, line 16-20, column 96, line 24-27, column 103, line 14-18, column 107, line 13-16, column 133, line 54-56, column 136, line 37-40, column 137, line 7-12, column 141, line 20-22, column 142, line 55-64, column 144, line 28-29, column 146, line 9-15, column 147, line 9-10, line 26-33, column 148, line 57-61	4-8, 12-16, 18-26, 30-37, 41-52, 60, 65-71, 78-93, 99-106, 109-112
Y	US 5,364,797 A (Olson, et al.) 15 Nov 1994 (15.11.1994) column 24, line 13-15, column 29, line 48-51	36-37, 51-52, 90

☐ Further documents are listed in the continuation of Box C.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"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

4 Sep 2008 (04.09.2008)

Date of mailing of the international search report

11 SEP 2008

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