(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 May 2010 (14.05.2010)

PCT

(10) International Publication Number
WO 2010/052342 A2

(51) **International Patent Classification**:
G01N 33/487 (2006.01) *G01N 33/543* (2006.01)

(21) **International Application Number**:
PCT/EP2009/064942

(22) **International Filing Date**:
10 November 2009 (10.11.2009)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
S2008/0900 10 November 2008 (10.11.2008) IE

(71) **Applicant** *(for all designated States except US)*: **NATIONAL UNIVERSITY OF IRELAND, MAYNOOTH** [IE/IE]; Co. Kildare (IE).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only)*: **COLLERAN, John** [IE/IE]; c/o National University of Ireland, Maynooth, Co. Kildare (IE). **HARLEY, Claire** [IE/IE]; c/o National University of Ireland, Maynooth, Co. Kildare (IE). **ALCOCK, Bernadette** [IE/IE]; c/o National University of Ireland, Maynooth, Co. Kildare (IE). **BRESLIN, Carmel** [IE/IE]; c/o National University of Ireland, Maynooth, Co. Kildare (IE). **FINNERTY, Niall** [IE/IE]; c/o National University of Ireland, Maynooth, Co. Kildare (IE).

(74) **Agents**: **LANE, Cathal Michael** et al.; Tomkins & Co., 5 Dartmouth Road, Dublin, 6 (IE).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) **Title**: CHEMICAL MESSENGER SENSOR

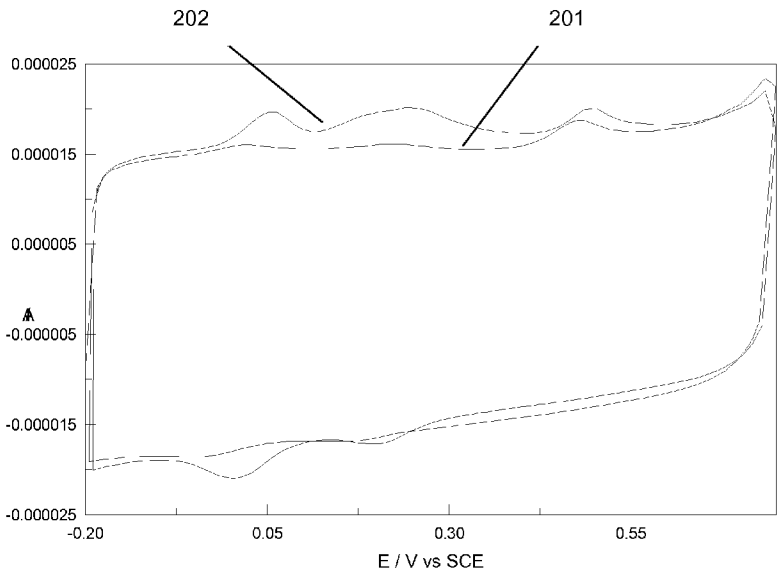

Figure 2

(57) **Abstract**: A sensor for the detection of chemical messengers is described herein. In particular, a sensor for the detection of serotonin is reported. Serotonin plays a pivotal role as a neurotransmitter in the modulation of a myriad of physiological responses including anger, aggression, mood, sleep, sexuality, and appetite. An electrode for detecting serotonin comprising a conducting or semiconducting substrate, and a polymer material on said substrate is disclosed. Said polymer comprises a conducting polymer doped with a cyclodextrin macrocycle. Suitable polymer materials include polypyrroles and polythiophenes, *e.g.* PEDOT. Suitable cyclodextrin macrocycles include anionic cyclodextrin macrocycles, for example sulfonated β-cyclodextrins (CDs). Also disclosed is a sensor capable of selectively detecting serotonin in the presence of ascorbic acid, epinephrine, norepinephrine and dopamine.

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Title

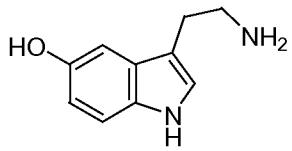
Chemical Messenger Sensor

Field of the Invention

[0001] A sensor for the detection of chemical messengers is described herein. In particular, a sensor for the detection of serotonin is reported. Methods of constructing sensors according to the present invention are also described. Suitable materials for the construction of such sensors are disclosed with a view to developing a sensor capable of real-time *in-vivo* serotonin monitoring.

Background to the Invention

[0002] Within the central nervous system a number of chemical messengers serve to maintain and regulate healthy brain function. Amongst these, serotonin (**1**) plays a pivotal role as a neurotransmitter in the modulation of a myriad of physiological responses including anger, aggression, mood, sleep, sexuality, and appetite.



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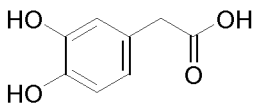
[0003] Implication of serotonin in the regulation of such a wide variety of physiological responses has led to its association in a corresponding high number of neurological diseases. In particular, imbalances in levels of serotonin in the brain are believed responsible for several psychiatric disorders, including depression. Thus, the ability to reliably monitor serotonin concentrations *in-vivo*, and on a real time scale would provide further valuable insight into the role of serotonin in the pathophysiology of the aforementioned disorders.

[0004] However, reliable *in-vivo* monitoring of serotonin is particularly challenging because it co-exists with a number of interfering species capable of oxidising at similar potentials. Interferant induced false positives ultimately lead to unreliable sensor readings. In particular, ascorbic acid (**4**) represents one of the key target interferants. Other common interferants such as uric acid (**5**), DOPAC (**2**) and homovanillic acid (**3**) are also problematic. The exclusion of these last two species is particularly advantageous as these are metabolites of dopamine, which are known to poison other biosensors reducing their sensitivity. As both dopamine and serotonin

neurotransmitters are active in the brain, there is a need to exclude cross metabolite contamination.



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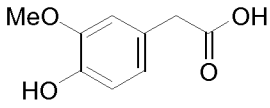

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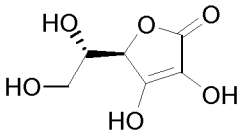

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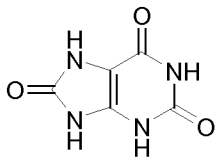

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[0005] The English language abstract of Japanese Patent Publication number 9127056 describes a sensor for selectively detecting serotonin in the presence of NO and NO_2. The sensor is comprised of three carbon fibre electrodes in a single assembly wherein an operational electrode is moved up and down upon a central axis. The English language abstract of Japanese Patent Publication number 3068858 discloses a simple arrangement for the electrochemical detection of serotonin. This publication is silent to the detection of serotonin the presence of interferants such as ascorbic acid.

[0006] Notwithstanding the foregoing, it would still be desirable to provide a biosensor capable of reliable real-time *in-situ*, *in-vivo* measurement of serotonin in the presence of interfering molecules such as ascorbic acid. Further still, such a biosensor must be able to function in the presence of metabolites without suffering the aforementioned problems associated with metabolite poisoning.

Summary of the Invention

[0007] The following abbreviations apply throughout the text and are used interchangeably with the non-abbreviated terms:

AA - ascorbic acid;

5-HT - serotonin;

CD - cyclodextrin;

β-CD - β-cyclodextrin; and

SCD - sulfonated cyclodextrin.

[0008] In one aspect, the present invention provides for an electrode for detecting serotonin comprising:

(i) a conducting or semi-conducting substrate; and

(ii) a polymer material on said substrate,

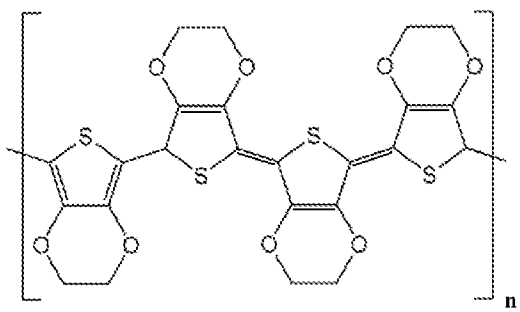
wherein said polymer comprises a conducting polymer doped with a cyclodextrin macrocycle.

[0009] As used herein, the term electrode encompasses both macroelectrodes having a diameter in excess of 1 mm and microelectrodes having a diameter less than 1 mm.

[0010] Desirably, the electrode of the present invention comprises a conducting substrate. As will be appreciated by a person skilled in the art the conducting substrate may comprise a metal selected from the group consisting of Pt, Ag, Au, Ru, Rh, Pd, Re, Os, Ir, Ti, Indium tin oxide (ITO) coated glass and combinations thereof. Further still, the conducting substrate may comprise a non-metallic conductor such as carbon fibres, graphite, glassy carbon, diamond, carbon paste and pyrolithic carbon electrodes, or boron doped diamond. Desirably, the conducting substrate comprises Au.

[0011] Preferably, the conducting polymer is a biocompatible conducting polymer. As used herein the term biocompatible is a reference to materials that are non-toxic to biological tissues.

[0012] The conducting polymer of the electrode of the present invention may be selected from the group consisting of polythiophenes, polypyrroles and combinations thereof. Desirably, the conducting polymer comprises a polythiophene. Suitably, the conducting polymer comprises PEDOT [polyethylenedioxythiophene] (**6**) wherein $n \geq 1$.



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[0013] Desirably, the cyclodextrin macrocycle comprises an anionic cyclodextrin macrocycle. Further desirably, the anionic cyclodextrin macrocycle comprises an anionic α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and combinations thereof. In a preferred embodiment the anionic cyclodextrin macrocycle comprises an anionic β-cyclodextrin.

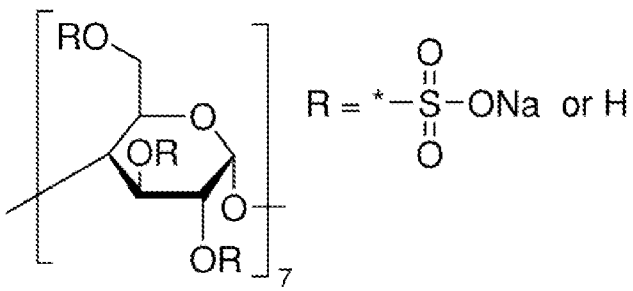
[0014] It is advantageous that the cyclodextrin macrocycle of the electrode of the present invention is anionic (negatively charged). At physiological pH metabolites known to poison prior art electrodes (for example DOPAC and homovanillic acid) are anionic species. As such the electrode construction of the present invention should not

be affected by these metabolites to the same extent, as the anionic cyclodextrin should repel these anionic metabolites.

[0015] The oxidative response of the film to serotonin is catalytic. That is, it results in the oxidation of serotonin occurring at a different potential than at the bare electrode (bare = gold, platinum, glassy carbon, *etc.*).

[0016] In a preferred embodiment the anionic cyclodextrin macrocycle comprises a sulfonated cyclodextrin macrocycle. Desirably, the sulfonated cyclodextrin macrocycle may comprise a sulfonated α-cyclodextrin, a sulfonated β-cyclodextrin, a sulfonated γ-cyclodextrin and combinations thereof. Further preferably, the anionic cyclodextrin macrocycle comprises a sulfonated β-cyclodextrin.

[0017] As used herein the term "sulfonated cyclodextrin macrocycle" refers to any cyclodextrin wherein one or more of the hydroxy groups of the glucopyranoside rings are sulfonated. Within the art the term is sometimes used interchangeably with sulfated cyclodextrin. Within this specification the terms sulfated cyclodextrin and sulfonated cyclodextrin are to be interpreted as one in the same provided the definition above is satisfied, *i.e.* having one or more of the hydroxy groups of the glucopyranoside rings sulfonated. For example, sulfonated β-cyclodextrin (**7**) is commercially available from Sigma-Alrich® as sulfated β-cyclodextrin.



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[0018] The electrode of the present invention may further comprise a deposit of a halogenated material on the polymer material. Suitable halogenated materials include fluorinated or chlorinated materials, such as perfluoro, perchloro and perfluorochloro polymers. In particular, of interest are halogenated ionomers such as a sulfonated tetrafluorethylene copolymer. One suitable commercially available material is Nafion® by DuPont. Nafion® may be considered a tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid copolymer (see for example CAS 31175-20-9).

[0019] The deposit of the halogenated material may form a coating around the electrode.

[0020] In a further aspect, the invention extends to a method of preparing an electrode for detecting serotonin comprising:

(i) providing a conducting or semi-conducting substrate;

(ii) providing an aqueous solution of monomeric precursor to a conducting polymer and an anionic cyclodextrin;

(iii) contacting said substrate and said aqueous solution; and

(iv) applying an electrical potential to provide an anionic cyclodextrin doped conducting polymer film on said substrate.

[0021] Desirably, the monomeric precursor to a conducting polymer comprises a monomeric precursor to a polythiophene, a monomeric precursor to a polypyrrole and combinations thereof. Further desirably, the monomeric precursor to a conducting polymer comprises a monomeric precursor to a polythiophene. Preferably, the monomeric precursor to a conducting polymer comprises ethylenedioxythiophene (EDOT), a precursor to polyethylenedioxythiophene (PEDOT).

[0022] The anionic cyclodextrin is incorporated into the PEDOT during polymerisation as a counter ion in order to neutralise the positive charge formed on the PEDOT chain during the oxidation of the monomer.

[0023] Desirably, in the method of the present invention the substrate comprises a conducting substrate. As will be appreciated by a person skilled in the art the conducting substrate may comprise a metal selected from the group consisting of Pt, Ag, Au, Ru, Rh, Pd, Re, Os, Ir, Ti, Indium tin oxide (ITO) coated glass and combinations thereof. Further still, the conducting substrate may comprise a non-metallic conductor such as carbon fibres, graphite, glassy carbon, diamond, carbon paste and pyrolithic carbon electrodes, or boron doped diamond. Desirably, the conducting substrate comprises Au.

[0024] Desirably, the cyclodextrin macrocycle comprises an anionic cyclodextrin macrocycle. Further desirably, the anionic cyclodextrin macrocycle comprises an anionic α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and combinations thereof. In one embodiment the anionic cyclodextrin macrocycle comprises an anionic β-cyclodextrin.

[0025] In a preferred embodiment of the method of the present invention the anionic cyclodextrin macrocycle comprises a sulfonated cyclodextrin macrocycle. Desirably, the sulfonated cyclodextrin macrocycle may comprise a sulfonated α-cyclodextrin, a sulfonated β-cyclodextrin, a sulfonated γ-cyclodextrin and combinations thereof.

Further preferably, the anionic cyclodextrin macrocycle comprises a sulfonated β-cyclodextrin.

[0026] The step of applying an electrical potential may comprise potentiostatic methods, and potentiodynamic methods such as cyclic voltammetry and combinations thereof.

[0027] The method of the present invention may further comprise the step of:

(v) applying a solution of a halogenated material to the electrode.

[0028] The electrode may be coated with a solution of the halogenated material. The electrode may be dipped into a solution of the halogenated material. For example, the electrode may be dipped between 5 and 10 times and allowed to dry between successive dippings. The halogenated material my be applied using the dip-coating or pre-coating methods described in the detailed description of the invention.

[0029] Suitable halogenated materials include fluorinated or chlorinated materials, such as perfluoro, perchloro and perfluorochloro polymers. In particular, of interest are halogenated ionomers such as a sulfonated tetrafluorethylene copolymer. One suitable commercially available material is Nafion® by DuPont. Nafion® may be considered a tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid copolymer (see for example CAS 31175-20-9).

[0030] The present invention further provides for a sensor for selective detection of serotonin in the presence of dopamine, epinephrine, norepinephrine, ascorbic acid and combinations thereof comprising:

an electrode comprising:

(i) a conducting or semi-conducting substrate; and

(ii) a polymer material on said substrate,

wherein said polymer comprises a conducting polymer doped with a sulfonated β-cyclodextrin macrocycle.

[0031] As used herein ascorbic acid comprises neutral ascorbic acid and the anionic derivative ascorbate. As will be appreciated by the skilled person, ascorbic acid when dissolved in water will dissociate into ascorbate anions and protons (in an amount proportional to the pKa of ascorbic acid).

[0032] Desirably, the sensor of the present invention comprises a conducting substrate. As will be appreciated by a person skilled in the art the conducting substrate may comprise a metal selected from the group consisting of Pt, Ag, Au, Ru, Rh, Pd, Re, Os, Ir, Ti, Indium tin oxide (ITO) coated glass and combinations thereof. Further still, the conducting substrate may comprise a non-metallic conductor such as carbon

fibres, graphite, glassy carbon, diamond, carbon paste and pyrolithic carbon electrodes, or boron doped diamond. Desirably, the conducting substrate comprises Au.

[0033] Preferably, the conducting polymer is a biocompatible conducting polymer. As used herein the term biocompatible is a reference to materials that are non-toxic to biological tissues.

[0034] The conducting polymer of the sensor of the present invention may be selected from the group consisting of polythiophene materials, polypyrrole materials and combinations thereof. Desirably, the conducting polymer comprises a polythiophene material. Suitably, the conducting polymer comprises PEDOT [polyethylenedioxythiophene].

[0035] The electrode of the sensor of the present invention may further comprise a deposit of a halogenated material on the polymer material. Suitable halogenated materials included fluorinated or chlorinated materials, such as perfluoro, perchloro and perfluorochloro polymers. In particular, of interest are halogenated ionomers such as a sulfonated tetrafluorethylene copolymer. One suitable commercially available material is Nafion® by DuPont. Nafion® may be considered a tetrafluoroethylene-perfluoro-3,6-dioxa-4-methyl-7-octenesulfonic acid copolymer (see for example CAS 31175-20-9).

[0036] The deposit of the halogenated material may form a coating around the electrode.

[0037] The electrode and sensor of the present invention provide for detecting serotonin in solution. As used herein the term solution comprises bodily fluids such as plasma, blood, extra-cellular fluid, *etc.* having serotonin dissolved therein.

[0038] Advantageously, the electrodes utilised in detecting serotonin levels have the potential to be miniaturised and conveniently placed in the living organism to give *in-vivo* data at the sub-second timescale. The electrode and sensor of the present invention may optimally sense serotonin at concentrations below 60 μM. When modified with a halogenated material such as Nafion® the electrode and sensor of the present invention may optimally sense serotonin at concentrations below 40 μM. At serotonin concentrations above these values the sensitivity of the electrode and sensor may decrease.

[0039] Advantageously, the electrode and sensor of the present invention provide for real-time measurement of serotonin, both *in-vivo* and *in-vitro*. Further still, the electrode and sensor of the present invention for detecting serotonin has the potential for *in-situ* monitoring.

[0040] Potential applications of the electrode and sensor of the present invention include the evaluation of test compounds on serotonin concentrations in the brain, and the resulting neurological response.

[0041] The relative simplicity with which these materials can be prepared, coupled with the excellent selectivity, high biocompatibility and ease of preparation shows that these novel materials have real potential in the sensing of Serotonin and are a significant improvement on the existing technologies. The materials utilised in the electrode are highly biocompatible (PEDOT is used in tissue engineering applications and cyclodextrins are used in drug delivery) and easy to prepare (10-min preparation time).

[0042] Where suitable, it will be appreciated that all optional and/or preferred features of one embodiment of the invention may be combined with optional and/or preferred features of another/other embodiment(s) of the invention.

Brief Description of the Drawings

[0043] Additional features and advantages of the present invention are described in, and will be apparent from, the detailed description of the invention and from the drawings in which:

[0044] Figure 1 illustrates electropolymerisation of PEDOT/cyclodextrin film at a Gold electrode according to the present invention. Potential cycled from -0.5 to +1.08 V vs SCE.

[0045] Figure 2 illustrates the cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode according to the present invention to $5x10^{-5}$ M 5-HT and a mixture of $5x10^{-5}$ M 5-HT & $5x10^{-4}$ M AA.

[0046] Figure 3 depicts the cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode according to the present invention to $5x10^{-5}$ M 5-HT & DA, and a mixture of $5x10^{-5}$ M 5-HT, DA & AA.

[0047] Figure 4 depicts the cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode according to the present invention to a mixture of 5-HT, AA, DA, EP and norEP.

[0048] Figure 5 illustrates the current response of 5.0 $x10^{-6}$ M 5-HT at the Au microelectrode using cyclic voltammetry.

[0049] Figure 6 illustrates the current response of AA using constant potential amperometry at a polymer modified platinum (Pt) microlectrode in the presence and absence of Nafion®.

[0050] **Figure 7** illustrates the current response of 5-HT using constant potential amperometry at a polymer modified platinum (Pt) microlectrode in the presence and absence of Nafion®.

Detailed Description of the Invention

[0051] It should be readily apparent to one of ordinary skill in the art that the examples disclosed herein below represent generalised examples only, and that other arrangements and methods capable of reproducing the invention are possible and are embraced by the present invention.

Preparation of the Electrode

[0052] The poor solubility of the 3,4-ethylene dioxythiophene (EDOT) monomer in aqueous solution, has led to the electropolymerisation of this monomer being predominantly performed in organic media.[1,2] Surfactants, such as sodium dodecyl sulphate (SDS), have been reported to improve the solubility of EDOT in aqueous and organic media.[3] In general, it has been communicated that a critical micellar concentration (cmc) of surfactant is required in solution if polymerisation of EDOT is to occur. Cyclodextrins have been used in place of surfactants,[4,5] owing to the ability of cyclodextrins to form a host guest interaction with EDOT, thus increasing the solubility of the EDOT monomer in water. In the example disclosed herein, sulfonated β-cyclodextrin (β-CD) was utilised as the dopant anion necessary for film formation to occur.

[0053] The films were electropolymerised onto gold electrodes from an aqueous solution of 0.1 M ethylenedioxythiophene and 0.01 M sulfonated β-cyclodextrin, sodium salt. Polymerisation was carried out by cycling the potential between -0.5 and 1.08 V/SCE at a scan rate of 50 mV s^{-1} for a total of three cycles.

[0054] The PEDOT/sulfonated β-cyclodextrin film properties vary depending on:

a) the EDOT:sulfonated β-CD solution concentrations (and ratio); and

b) the polymerisation technique utilised - when cyclic voltammetry is utilised the following parameters can be modified to vary the PEDOT/sulfonated β-cyclodextrin film properties;

   i) the upper (anodic) potential of the voltammetric sweep used when fabricating the polymer film. This upper potential is important for system optimisation; and

   ii) the sweep rate.

[0055] The conditions resulting in optimal serotonin sensing comprise:

- a polymerisation solution of 0.1M EDOT:0.01M sulfonated β-CD; this 10:1 ratio is important;
- cyclic voltammetry (CV) is important to ensure that a homogeneous thin film is formed;
- sweeping from -0.5 to +1.08 V vs SCE at a scan rate of 50 mV s^{-1}; and
- three electropolymerisation cycles to form a thin but a homogeneous film.

[0056] All scans were completed on a Solartron 1285 potentiostat. The data provided herein and in the figures were obtained using cyclic voltammetry unless otherwise stated.

[0057] For example, in **Figure 1** we see a cyclic voltammagram of three electropolymerisation cycles of a solution of 0.1M EDOT:0.01M sulfonated β-CD. Irreversible oxidation, *i.e.* electropolymerisation of the monomer occurs at approximately 0.8V and leads to film formation (101). No peak in the reverse sweep direction indicates that this is essentially an irreversible process.

Serotonin (5-HT) Detection

Detection at the PEDOT/CD film:

[0058] The cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode to $5x10^{-5}$ M 5-HT (trace 201) and a mixture of $5x10^{-5}$ M 5-HT & $5x10^{-4}$ M AA (trace 202) is shown in shown in **Figure 2**. The main 5-HT oxidation peak is observed at ~0.48V in the 5-HT only trace 201. In trace 202 the 5-HT peak is measured in the presence of AA. In the presence of AA the 5-HT peak is shifted slightly to ~0.5V. Thus, the electrode array of the present invention comprising the PEDOT/sulfonated β-CD film can selectively detect 5-HT in the presence of AA.

Interference of Dopamine (DA) with the Serotonin (5-HT) oxidation signal:

[0059] As dopaminergic and serotoninergic neurons are active within close proximity in the animal/human brain desirably the electrode array of the present invention should be capable of detecting 5-HT in the presence of DA. **Figure 3** shows the cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode according to the present invention to $5x10^{-5}$ M 5-HT & DA (lower trace 301), and a mixture of $5x10^{-5}$ M 5-HT, DA & AA (upper trace 302). In 301, notwithstanding an overlap between the two peaks, we observe separate signals for DA 303 and 5-HT 304. The oxidation peaks are at ~0.41V and ~0.48V respectively. Trace 302 in the

presence of AA, DA and 5-HT illustrates separate discernable peaks for AA 305, DA 306 and 5-HT 307.

[0060] Gratifyingly, the electrode comprising the PEDOT/sulfonated β-CD film according to the present invention was capable of selectively detecting all three species as independent peaks.

Selective Detection of Serotonin in the presence of Ascorbic Acid (AA), Dopamine (DA), Epinephrine (EP) and Norepinephrine (norEP):

[0061] Similarly, selective detection of 5-HT in the presence of AA, DA, EP and norEP was evaluated. **Figure 4** shows the cyclic voltammetric response of the PEDOT/sulfonated β-CD film on a gold electrode according to the present invention to a mixture of $1.67x10^{-5}$ M 5-HT, $2x10^{-4}$ M AA, $2x10^{-5}$ M DA, $1x10^{-5}$ M EP and $6.67x10^{-6}$ M norEP. The signals labelled 401 at ~0.035V, ~0.15V & ~0.5V correspond to 5-HT. Signal 402 at ~0.2V is from ascorbic acid, and signal 403 at ~0.42V is a mixture of DA, EP and norEP.

[0062] Figure 4 clearly illustrates that the gold electrode comprising the PEDOT/sulfonated β-CD film according to the present invention is capable of selectively detecting 5-HT in the presence of AA, DA, EP and norEP.

Preparation of a Microelectrode for Sensing Serotonin

[0063] For utility *in-vivo* microelectrodes are desirable on account of their smaller size. Suitably, the microelectrode should have a diameter less than 200 µm. Preferably, the microelectrode selected should have a diameter of less than 180 µm. References to diameter are inclusive of any additional layers or deposits applied to the electrode surface.

[0064] A modified gold microelectrode having a diameter of 75 µm (0.075 mm) was prepared as follows:

A PEDOT/sulfonated β-cyclodextrin film was electropolymerised onto a gold electrode of 75 µm diameter from an aqueous solution of 0.1 M ethylenedioxythiophene and 0.01 M sulfonated β-cyclodextrin, sodium salt. Polymerisation was carried out by cycling the potential between -0.5 and 1.08 V/SCE at a scan rate of 50 mV s^{-1} for a total of three cycles.

[0065] Figure 5 illustrates the current response of 5.0 $x10^{-6}$ M 5-HT at the Au microelectrode using cyclic voltammetry. The full sweep of the cyclic voltammegram is shown in the top right hand corner. The main image is an exploded view of the region

of the cyclic voltammegram showing the current response 501 of the 5-HT. The peak 501 corresponding to oxidation of 5-HT is observed between 0.40 V and 0.50 V.

Microelectrodes Coated with Nafion®

[0066] A PEDOT/sulfonated β-cyclodextrin film was coated on to a platinum (Pt) microelectrode using the same method disclosed for the gold microelectrode *supra*.

[0067] Nafion®, a perfluorinated polymer, may be applied to electrodes in order to establish selectivity against electroactive interferents. Methods of doing this include the dip-coat method and the pre-coat method.

[0068] The pre-coat method involves placing a fixed volume (*e.g.* 5 ml) of Nafion® onto a watch glass using a syringe. The Nafion® droplet is allowed to air dry at room temperature for 5 minutes. After the solvent (5% Nafion® dissolved in aliphatic alcohols) from the initial droplet has evaporated, further individual droplets are placed on top of the original droplet using the same procedures previously outlined for the initial drop. What results from solvent evaporation is a concentrated layer of Nafion® on the watch glass.

[0069] After 1-5 drops (called 1-5 pre-coats) have been placed onto the watch glass, a final drop of Nafion® is placed onto the concentrated pre-coat of Nafion. The active surface of the electrode is then dipped into the Nafion® concentrated layer. The electrode is then removed immediately from the concentrated layer of Nafion® and let air dry at room temperature for 2 minutes. The purpose of the fresh Nafion® droplet is to adhere the concentrated Nafion® layer to the electrode. The electrode may optionally be annealed to help drying. The electrode can be coated again using the same procedure if desired. This pre-coat fabrication can be carried out numerous times.

[0070] In the experiments below Nafion® was deposited onto the surface of the PEDOT/sulfonated β-cyclodextrin film using the dip-coating method. The PEDOT/sulfonated β-cyclodextrin film was dipped into the Nafion® solution and then allowed to dry for 5 minutes before dipping again. In total 10 dips were performed.

[0071] Figure 6 illustrates the current response of AA at the Pt PEDOT/sulfonated β-cyclodextrin modified microelectrode using constant potential amperometry. Concentration of AA is plotted on the X-axis and Current (I) measured is plotted on the Y-axis. Trace 601 illustrates the response of AA at the Pt-PEDOT/sulfonated β-cyclodextrin microelectrode. The current measured increases with increasing AA concentration. Trace 602 illustrates the response of AA at the Pt-PEDOT/sulfonated β-cyclodextrin/Nafion® microelectrode. In the presence of Nafion® minimal AA is

oxidised at the electrode, thus no change in current is registered. At high AA concentrations a very low current is observed. Thus indicating that Nafion® is hindering oxidation of AA at the electrode.

[0072] Figure 7 illustrates the current response of 5-HT at the Pt PEDOT/sulfonated β-cyclodextrin modified microelectrode using constant potential amperometry. Concentration of 5-HT is plotted on the X-axis and Current (I) measured is plotted on the Y-axis.

[0073] Trace 701 illustrates the response of 5-HT at the Pt-PEDOT/sulfonated β-cyclodextrin microelectrode. In the absence of Nafion®, it is evident that 5-HT concentrations above 60 μM result in no change in the current measured. Thus, a threshold 5-HT concentration value has been reached, beyond which no further oxidation of 5-HT at the electrode is observable.

[0074] Trace 602 illustrates the response of 5-HT at the Pt-PEDOT/sulfonated β-cyclodextrin/Nafion® microelectrode. For the Nafion® modified microelectrode a lower threshold 5-HT concentration of 40 μM is observable. For 5-HT concentrations greater than 40 μM the measured current appears to decrease. *In-vivo* concentrations of 5-HT are significantly lower than 40 μM and the issues surrounding the threshold concentration values of 5-HT should not be problematic. In conclusion, at concentrations less than or equal to 40 μM of 5-HT, the Nafion/PEDOT/ sulfonated β-cyclodextrin modified microelectrode shows efficient qualities for the detection of 5-HT.

[0075] Microelectrode analyses were performed utilising AD Instruments Powerlab 8/30 in conjunction with an ACM Four Channel Biostat.

[0076] The words “comprises/comprising” and the words “having/including” when used herein with reference to the present invention are used to specify the presence of stated features, integers, steps or components but do not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

[0077] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

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Claims

1. An electrode for detecting serotonin comprising:

   (i) a conducting or semi-conducting substrate; and

   (ii) a polymer material on said substrate,

   wherein said polymer comprises a conducting polymer doped with a cyclodextrin macrocycle.

2. An electrode according to Claim 1 wherein the substrate comprises a conducting substrate.

3. An electrode according to Claim 1 or 2 wherein the conducting substrate comprises Au.

4. An electrode according to any preceding Claim wherein the conducting polymer is selected from the group consisting of polythiophenes, polypyrroles and combinations thereof.

5. An electrode according to Claim 4 wherein the conducting polymer comprises a polythiophene.

6. An electrode according to Claim 5, wherein the conducting polymer comprises polyethylenedioxythiophene.

7. An electrode according to any preceding Claim wherein the cyclodextrin comprises an anionic cyclodextrin.

8. An electrode according to Claim 7, wherein the anionic cyclodextrin comprises an anionic α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and combinations thereof.

9. An electrode according to Claim 7 or 8, wherein the anionic cyclodextrin comprises an anionic β-cyclodextrin.

10. An electrode according to Claim 9 wherein the anionic β-cyclodextrin comprises a sulfonated-β-cyclodextrin.

11. An electrode according to any preceding Claim further comprising a deposit of a halogenated material on the polymer material.

12. A sensor for selective detection of serotonin in the presence of dopamine, epinephrine, norepinephrine, ascorbic acid and combinations thereof comprising:

an electrode comprising:

(i) a conducting or semi-conducting substrate; and

(ii) a polymer material on said substrate,

wherein said polymer comprises a conducting polymer doped with a sulfonated β-cyclodextrin macrocycle.

13. A sensor according to Claim 12 comprising a conducting substrate.

14. A sensor according to Claim 13 wherein the conducting substrate comprises Au.

15. A sensor according to any preceding Claim wherein the conducting polymer is selected from the group consisting of polythiophene materials, polypyrrole materials and combinations thereof.

16. A sensor according to Claim 15 wherein the conducting polymer comprises a polythiophene material.

17. A sensor according to Claim 16, wherein the conducting polymer comprises polyethylenedioxythiophene.

18. A sensor according to Claims 12 to 17 wherein the electrode further comprises a deposit of a halogenated material on the polymer material.

19. A method of preparing an electrode for detecting serotonin comprising:

(i) providing a conducting or semi-conducting substrate;

(ii) providing an aqueous solution of a monomeric precursor to a conducting polymer and an anionic cyclodextrin;

(iii) contacting said substrate and said aqueous solution; and

(iv) applying an electrical potential to provide an anionic cyclodextrin doped conducting polymer film on said substrate.

20. A method according to Claim 19 wherein the monomeric precursor to a conducting polymer comprises a monomeric precursor to a polythiophene, a monomeric precursor to a polypyrrole and combinations thereof.

21. A method according to Claim 20 wherein the monomeric precursor to a conducting polymer comprises a monomeric precursor to a polythiophene.

22. A method according to Claims 19 to 21 wherein the monomeric precursor to a conducting polymer comprises ethylenedioxythiophene.

23. A method according to any preceding Claim comprising providing a conducting substrate.

24. A method according to Claim 23 wherein the conducting substrate comprises Au.

25. A method according to any preceding Claim wherein the anionic cyclodextrin comprises an anionic α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and combinations thereof.

26. A method according to Claim 25 wherein the anionic cyclodextrin comprises an anionic β-cyclodextrin.

27. A method according to Claim 26 wherein the anionic β-cyclodextrin comprises a sulfonated-β-cyclodextrin.

28. A method according to Claims 19 to 27 further comprising the step of:

(v) applying a solution of a halogenated material to the electrode.

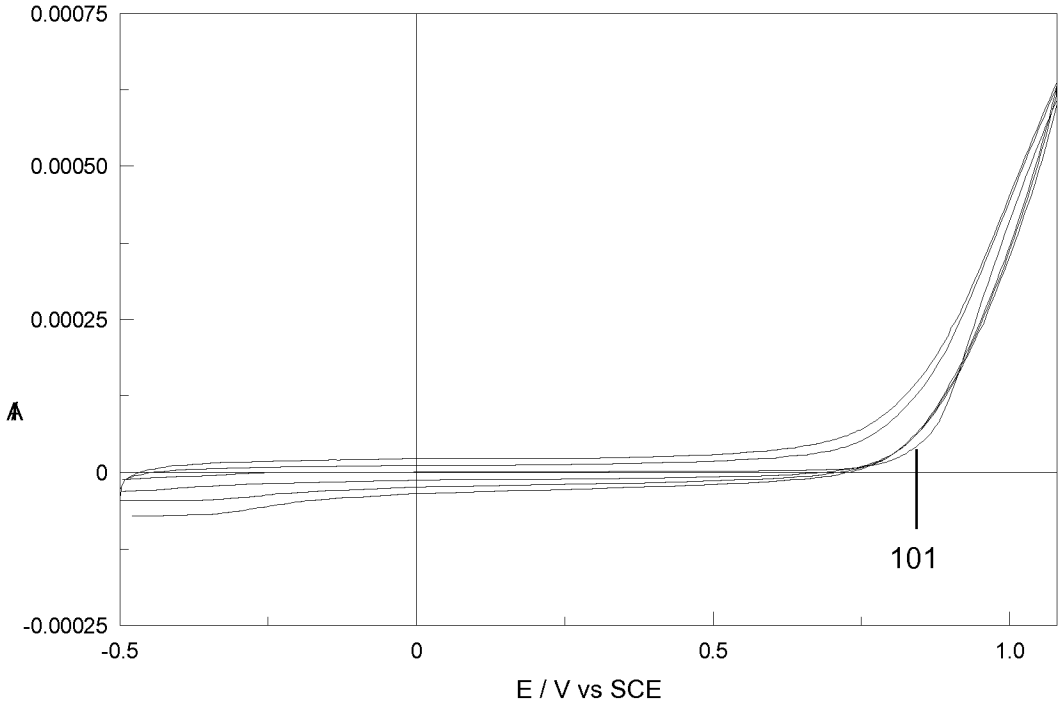


Figure 1

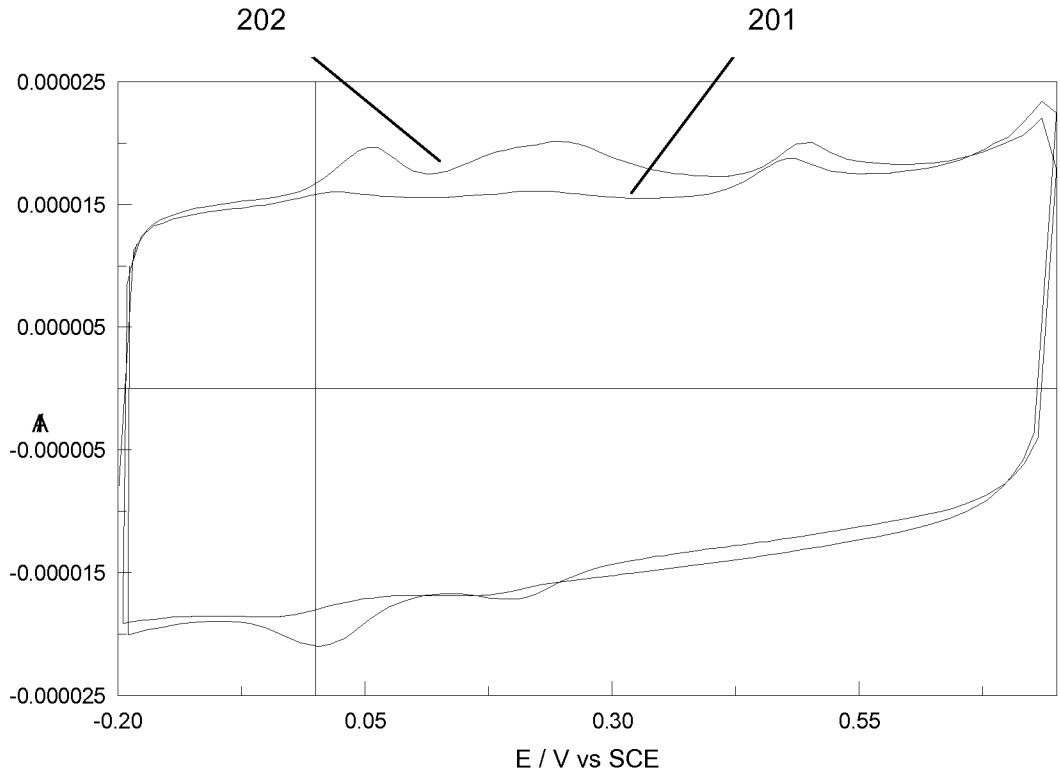


Figure 2

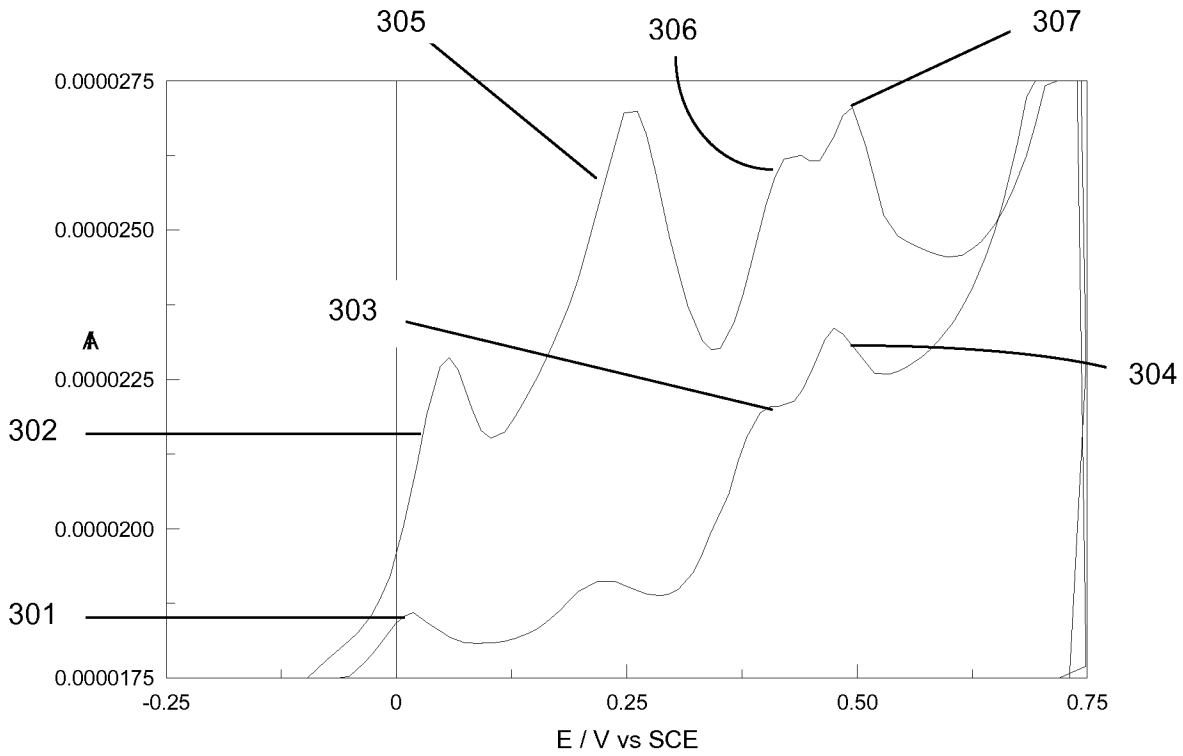


Figure 3

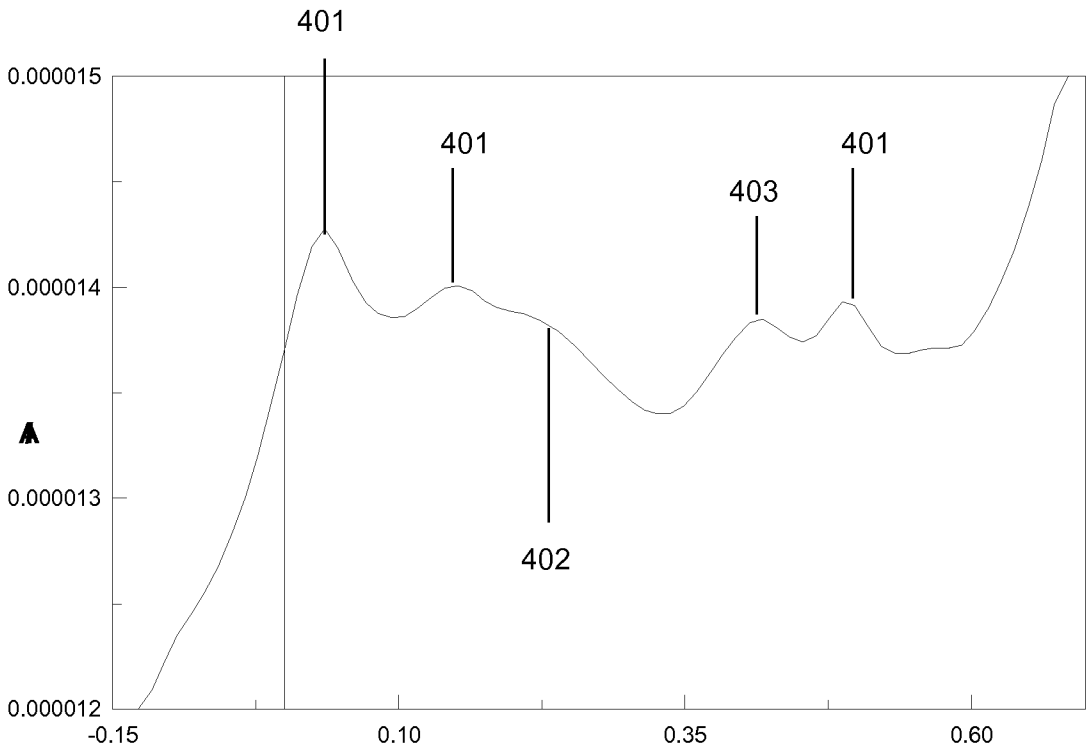
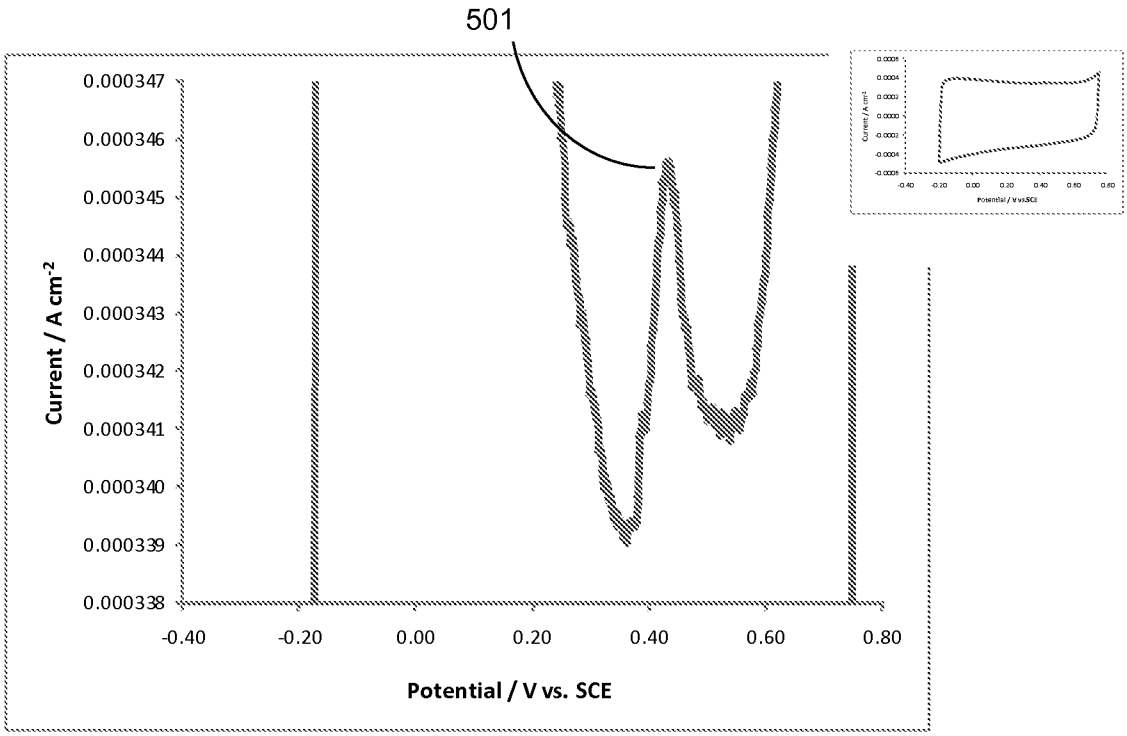


Figure 4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0.000341
0.000340
0.000339
0.000338
Current / A cm-2
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-0.20
0.00
0.20
0.40
0.60
0.80
Potential / V vs. SCE

Figure 5

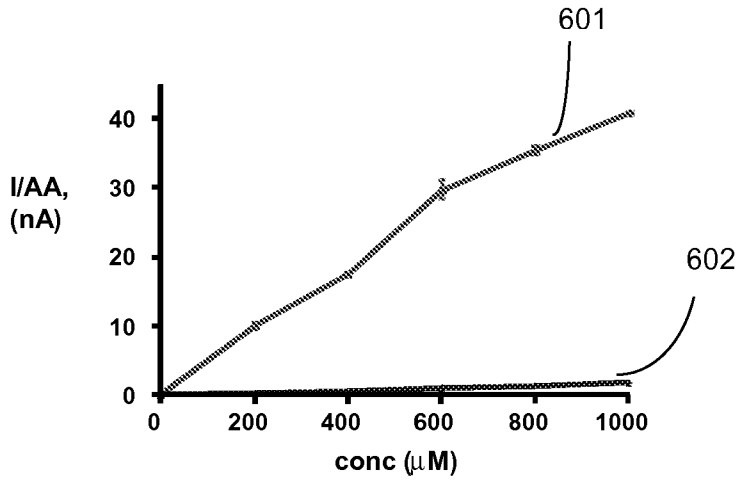

Figure 6

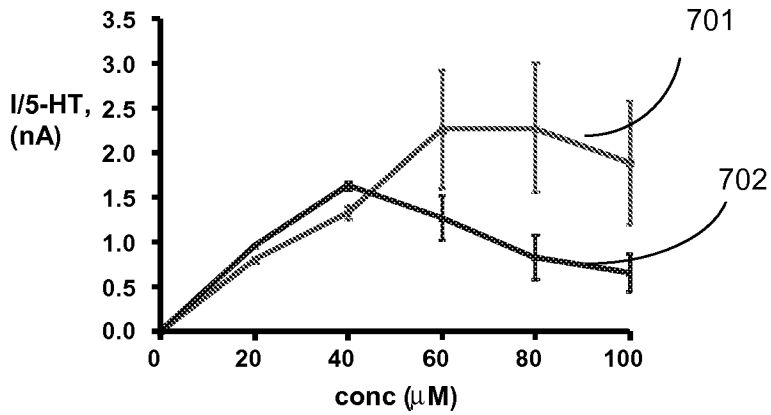

Figure 7